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SHORT COMMUNICATIONS

Papaverine-induced changes in cultured human melanoma cells*

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CHANGES in morphology, pigment production and growth rates follow exposure of cultivated mouse melanoma cells[1] or fish melanoma explants[2] to dibutyryl cyclic AMP (dbc-AMP). We have extended these observations to a human melanoma cell line (RPMI 7931) originally established by Dr. George E. Moore of Rosewell Park Memorial Institute, and maintained free of mycoplasma contamination by Dr. Jorgen Fogh at the Memorial Sloan–Kettering Cancer Center.

In our studies, the cells were exposed to N^6,$O^{2'}$-dibutyryl adenosine-3',5'-monophosphate (dbc-AMP), sodium butyrate (NaB), paperverine and 5-trifluoromethyl-2'-deoxyuridine (F3-TdR). Sodium butyrate was tested because of its potential contribution as part of the dbc-AMP molecule. Papaverine is a phosphodiesterase inhibitor known to cause accumulation of endogenous cyclic nucleotides[3] and observed to produce changes in cell morphology similar to dbc-AMP in another ectodermally derived tumor, the human neuroblastoma.[4] F3-TdR, a fluorinated pyrimidine and thymidylate synthetase inhibitor,[5] was tested because of its similarity to bromo-deoxyuridine, which is known to affect pigment production in murine melanoma cells, and because of interest in its potential use in a clinical setting.[7]

The cells were grown in 25-ml vol plastic flasks (Falcon Plastics, Oxnard, Calif.) containing 7 ml Eagle's medium with added glutamine, non-essential amino acids, penicillin, streptomycin, 15 per cent heat-inactivated fetal bovine serum and 2 mM tyrosine. Cultures were incubated at 37° in a 5% CO_2 atmosphere. Stock cultures were grown to confluence and refed with fresh media; 24 hr later the cells were detached with 0·25% trypsin and the number and size distribution of cells/flask were determined with a Coulter Counter (model ZBI). Replicate flasks were inoculated with 10^6 tumor cells in 7 ml of medium containing varying concentrations of dbc-AMP, or sodium butyrate, or papaverine, or F3-TdR. After 5 days, the medium and floating debris were withdrawn from each flask and replaced with drug-free medium. On day 6, cells were detached with trypsin, washed twice with phosphate buffered saline (PBS), enumerated and reinoculated in aliquots of 10^6 cells in fresh drug-free medium so as to eliminate the effect of contact inhibition in dense cultures.

In two replicate cultures, additional papaverine was added. Pigment production was measured by the method of Silagi and Bruce[6] in replicate flasks. Six days later (following medium replenishment on the previous day), the cells were harvested and counted. Thus, the cells were exposed to drugs for 5 days, washed, and observed in drug-free medium an additional 7 days for morphological and growth changes.

The characteristic morphology of this cell line includes epitheloid, bi-polar and oligodendritic cells (Fig. 1). After 24- to 48-hr exposure to 0·5 mM dbc-AMP, or 0·039 mM papaverine (Fig. 2) or 0·01 mM F3-TdR, virtually all attached cells developed elongated dendritic processes. An increase in cell volume was noted in a significant proportion of the cell population after exposure to 0·01 mM F3-TdR (Fig. 3). Lesser or no changes were observed at lower concentrations of these three compounds. The morphologic alterations appeared prior to any grossly observable evidence of growth inhibition. Cells exposed to dbc-AMP and papaverine reverted to pretreatment morphology within 24 hr after replacement with fresh medium. F3-TdR cells retained their changed morphology during a 21-day period in which the cells were washed three times with PBS, fresh medium, and detached twice with trypsin for reinoculation and growth studies. After the third transfer, the F3-TdR-treated cells reverted to their original morphologic state. This prolonged retention of morphologic changes after treatment with F3-TdR contrasted sharply with dbc-AMP- and papaverine-treated cells. Sodium butyrate (0·5 or 1·0 mM) did not affect cell morphology.

These observations of readily reversible morphologic changes in dbc-AMP- and papaverine-treated human melanoma cells are similar to changes seen with mouse fibroblasts,[8] and contrast with mouse melanoma cells exposed to dbc-AMP[1] where morphologic changes are less reversible. There are cell specific differences in response to cyclic nucleotides, however, i.e. in a separate study, fibroblasts established from the bone marrow of a child with neuroblastoma* and cultured under identical conditions to the melanoma cells in this study were neither growth inhibited nor had morphological changes after exposure to 0·5 or 1·0 mM dbc-AMP.

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* L. Helson, unpublished observations.

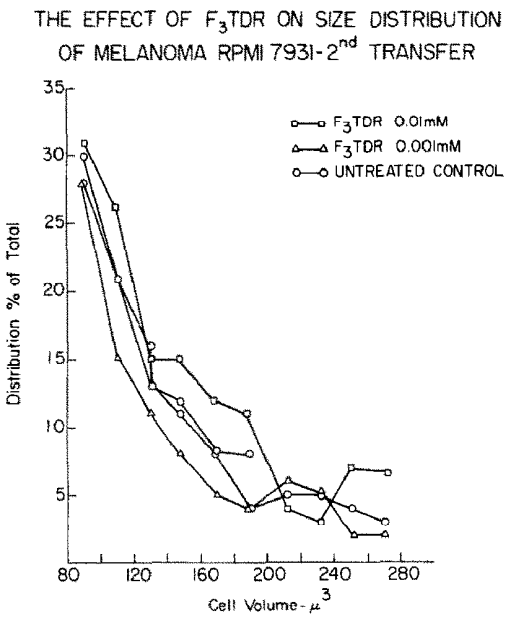

FIG. 3. Significant increase ($P < 0.05$) in the proportion of cells ranging from 140 to 180 μ^3 after treatment with 0·01 mM F3-TdR.

For all substances tested, melanoma cell growth inhibition was concentration dependent, and the dose proportionate decrease in growth rate persisted in the washed and reinoculated cells. A similar (72-hr) persistent growth lag was recently reported in a study of the effects of dbc-AMP on a sensitive cell line of strain L mouse fibroblasts.[9] Re-exposure of once treated and washed cells was carried out at one concentration of papaverine (0·39 mM) and produced only modest further inhibition of cell proliferation (Fig. 4). These results are constant with several drug toxicity models which include the following: (1) residual binding of drug-substance may persist for some cell generations, (2) some of the metabolic changes produced by the drug may persist in its absence, and (3) relatively resistant cells may be present in the control

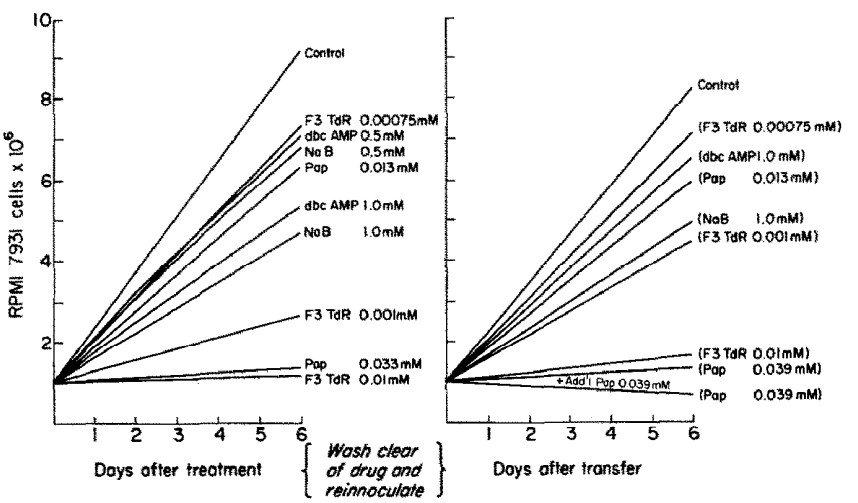

FIG. 4. Growth effects of various drugs on the growth of cultured melanoma (RPMI 7931) cells. F3TdR = 5-trimethyl fluoro-2-deoxyuridine; dbc-AMP N^6,O^2-dibutyryl adenosine 3′,5′ monophosphate; NaB, sodium butyrate; Pap = papaverine.

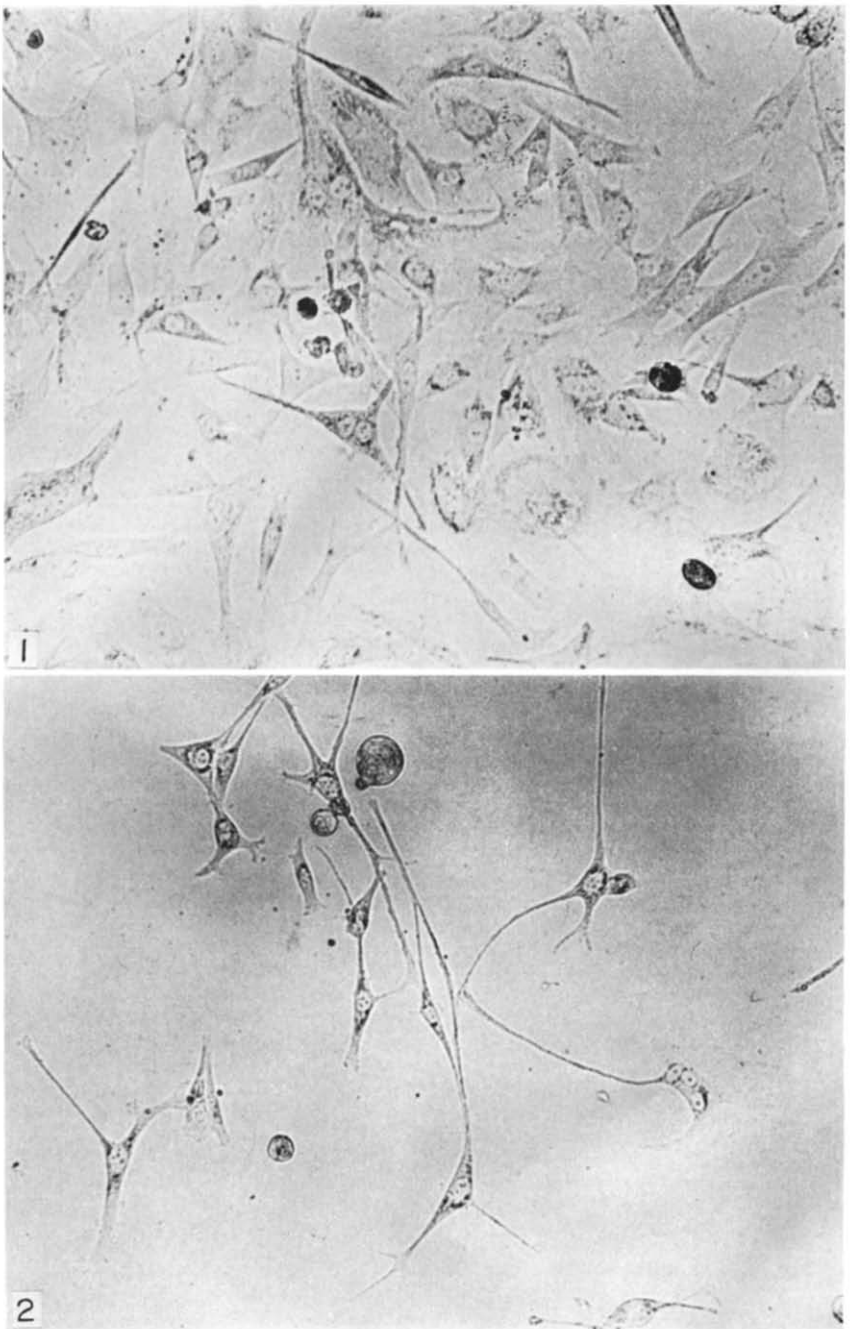

FIG. 1. Untreated melanoma cells 6 days after inoculation of 10^6 cells. Phase contrast ×100.

FIG. 2. Melanoma cells 6 days after incubation with 0·039 mM papaverine. Both growth inhibition and morphologic changes are evident. Phase contrast ×100.

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population that are selected by drug exposure and are characterized by a slower growth rate or poorer plating efficiency than the original population. Further studies will be required to support or nullify these possibilities. The growth inhibitory activity of the drugs was not unexpected. Dbc-AMP has already been shown to inhibit growth of cultured neuroblastoma cells and lymphocytes;[4] sodium butyrate was first shown to affect cultured human cell lines (skin, lung, HeLa strain L) at a concentration of 1·0 μg/ml.[10] The mechanism of action of sodium butyrate-induced growth inhibition of these melanoma cells remains unclear. Growth inhibition of murine and human neuroblastoma cells by sodium butyrate has also been observed; however, morphologic changes occur in human but not murine neuroblastoma cells.[4,11] This may be a manifestation of the expressive properties of the particular cell line under study rather than a specific pharmacologic action.

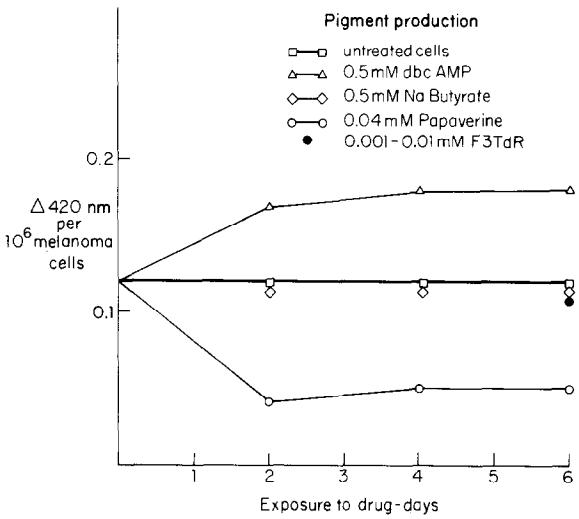

FIG. 5. Drug effects upon melanoma cells; each point represents the mean values from two replicate flasks.

Pigment production was increased by exposure to 0·5 mM dbc-AMP after 2 days and paralleled morphologic changes. Neither sodium butyrate, F3-TdR nor papaverine induced pigment changes (Fig. 5). Cell contact, considered to be a promoter of pigment formation in the mouse melanoma,[12] was not an operative factor in these experiments, wherein cultures with similar extents of growth inhibition by 0·5 mM sodium butyrate or 0·5 mM dbc-AMP (Fig. 5) had decreased intercellular contact, but only the dbc-AMP-treated cells had pigment induced.

F3-TdR has had both clinical and pharmacological study and is known to affect a variety of different mammalian cells.[5] Its morphological and growth inhibitory effects in human melanoma cells appear to be similar to that produced by BUdR[6] or papaverine.

These data indicate that human melanoma cells are similar to murine melanoma cells in their growth, morphology and pigmentary susceptibility to change after exposure to a cyclic nucleotide, a phosphodiesterase inhibitor and a fluorinated pyrimidine. The mechanism of sodium butyrate inhibition remains obscure. The human tumor cells have distinctive characteristics exhibited by prolonged growth inhibition after exposure to these agents and singular prolonged morphologic changes after exposure to F3-TdR. Further investigation with this tumor model may permit the development and eventual utilization of phosphodiesterase inhibitors such as papaverine and fluorinated pyrimidines in a clinical setting.

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Effect of *p*-chlorophenylalanine and L-phenylalanine on deoxyribonucleic acid and protein content of developing rat cerebellum

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TREATMENT of laboratory animals with *p*-chlorophenylalanine (*p*-CPA), an irreversible phenylalanine and tryptophan hydroxylase inhibitor,[1,2] has been used as an experimental model of phenylketonuria.[3,4] However, supplemental L-phenylalanine is necessary to increase plasma and tissue phenylalanine levels significantly.[5] A consistent observation in neonatal animals undergoing this treatment has been an irreversible reduction in brain weight.[6,7] The cause of the reduced brain weight is unknown, but its irreversibility suggests a decrease in the number of brain cells. DNA concentration is constant within diploid cells in a given species[8] and has been used as a measure of cell number.[9] Protein to DNA ratios indicate cell size.[9] This paper reports the effect of *p*-CPA and phenylalanine treatment on changes of the cerebellum in developing rat pups and in adult rats rehabilitated after early postnatal treatment. Brain growth was examined by measuring weight changes and DNA and protein concentrations.

TABLE 1. EFFECT OF *p*-CHLOROPHENYLALANINE AND L-PHENYLALANINE TREATMENT ON BODY AND CEREBELLUM WEIGHTS OF DEVELOPING RAT*

Age (days)	Body wt (g)		Cerebellum wt (mg)	
	Control	Experimental	Control	Experimental
9	18·3 ± 3·0 (6)	16·1 ± 1·0 (6)	91 ± 15	68 ± 13†
15	27·3 ± 5·1 (14)	20·5 ± 4·1† (14)	152 ± 19	112 ± 17†
21	46·7 ± 4·1 (13)	31·8 ± 4·3† (15)	190 ± 13	130 ± 16†
154	254·0 ± 25·2 (6)	268·5 ± 33·6 (6)	297 ± 16	218 ± 23†

* For details of treatment see text. Numbers of animals are given in parentheses.
† $P < 0.01$.