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Research Letters

The specific inhibitor of protein kinase C, 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H7), induces morphological change and cell differentiation of human neural crest-derived cell lineages

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It has been proved that inhibition of protein kinase C by 1-(5-isoquinolinylsulfonyl)-1-methylpiperazine (H7) induces morphological differentiation in murine neuroblastoma (nb) cell. Here we report that H7 is also active on human nb cell lines. The human nb cell had originally neuroblast-like (N) or intermediate (I) morphology. N and I type are thought to represent different stages of neuroblastoma differentiation. Neurite outgrowth was observed in both N and I type morphology treating the cells with 7, 14 or 28 μM of H7. The results confirm previous observations and show that inhibition of PKC by H7 also promotes neuronal differentiation in human cell line variants.

Neural differentiation; Protein kinase C; 1-(5-Isoquinolinylsulfonyl)-2-methylpiperazine (H7); Human neuroblastoma

1. INTRODUCTION

Neuroblastoma cells can be induced to differentiate in vitro using natural or chemical agents [1–4]. Neural cell differentiation is accompanied by outgrowth of neurite-like structures which form a complex network.

Recently, Minana et al. [5] reported that murine neuro-2a cells can be fully differentiated in the presence of 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H7), a specific inhibitor of protein kinase C (PKC). They suggest a role of PKC in the control of neural differentiation.

Here, we report our experience concerning the treatment with H7 of three human neuroblastoma cell lines showing neuroblast (N) or intermediate (I) morphology [6]. Neurites were detected either in N and I type cells. This observation extends knowledge obtained from previous results [5,7] on the role of PKC in neuroblastoma cell differentiation and shows that specific inhibition of PKC produces neurite outgrowth of diverse human neuroblastoma cell variants.

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2. MATERIALS AND METHODS

2.1. *Neuroblastoma cell lines*

SK-N-BE2(C), I-type; Lan-1 and Lan-5, N-type neuroblastoma cells (kindly provided by V. Ciccarone, NCI, Frederick) were cultured as previously described [2].

2.2. *Treatment of the cells with H7*

Dose-response curve was assessed using the following final concentrations of H7: 3.5, 7, 14 and 28 μM dissolved in ethanol and added to the culture at time: 0, 48 and 96 h. Control cells were treated with ethanol. Every 48 h the medium was changed and H7 added again. Cell viability was checked by the Trypan blue exclusion test. Neurite formation was observed by phase contrast light microscope and photographed.

3. RESULTS AND DISCUSSION

Neurite outgrowth accompanied by clear morphological differentiation was observed in human neuroblastoma cell lines SK-N-BE(2)C, Lan-1 and Lan-5 treated with 28 μM H7. Fig. 1 shows the morphology of the cells after 7 days of treatment with 28 μM of H7. Dose-response curve (data not shown) indicated that Lan-1 cells were more sensitive to H7 activity (7 μM was sufficient to produce neurites) than SK-N-BE(2)C which needed 28 μM (Table I). At a concentration of 28 μM, H7 induced morphological differentiation of all three cell lines irrespective of their basal morphology, neuroblast- or intermediate-type. N-type cell is small, round and presents some neurite-

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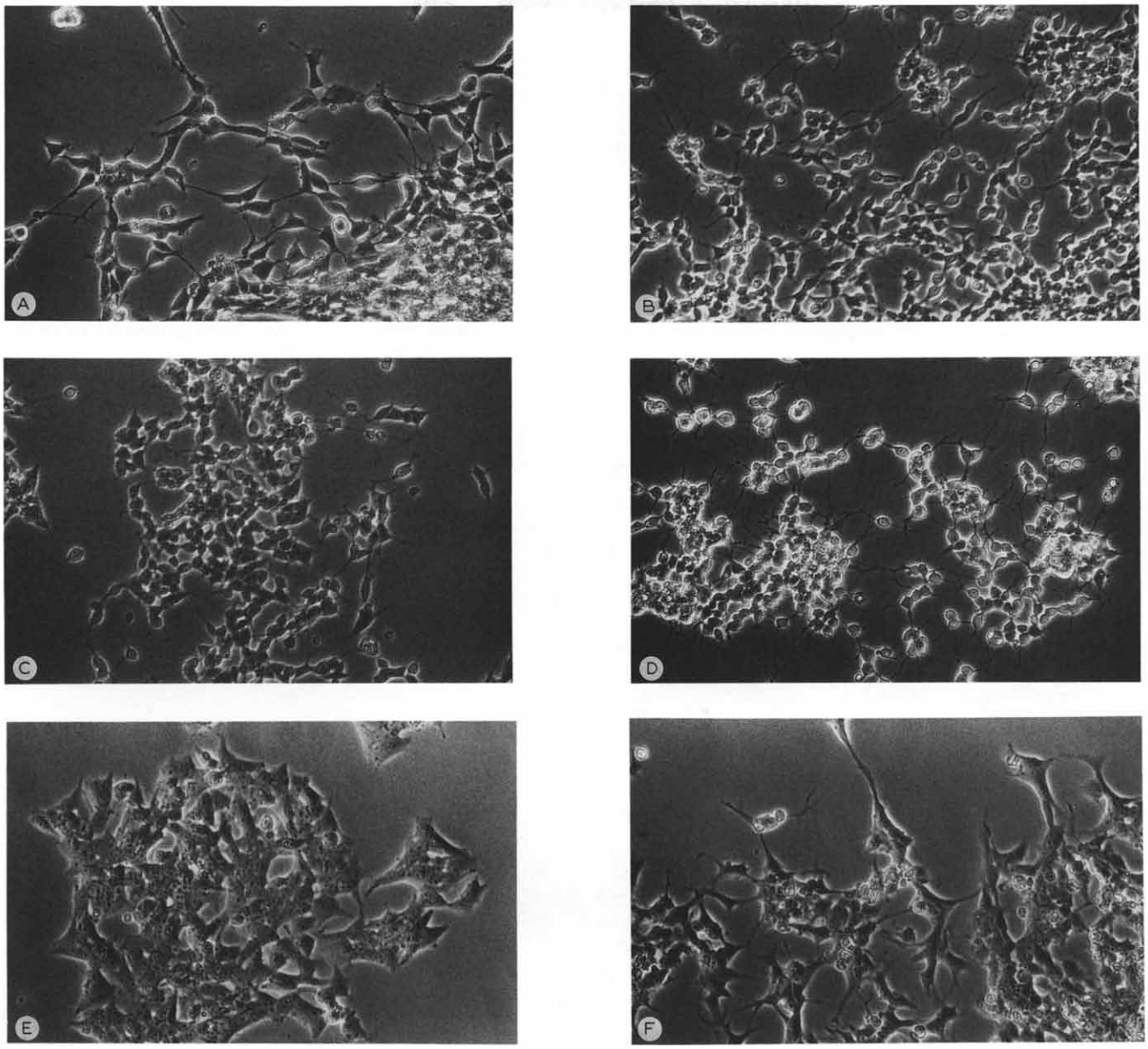

Fig. 1. Neurite outgrowth and morphological differentiation of human neuroblastoma cell variants treated with 28 μM of H7. LAN-1 medium (A), H7 (B); LAN-5 medium (C), H7 (D), both cell lines are N-type morphology. SK-N-BE(2)C medium (E), H7 (F), I-type morphology. The cells were photographed with a phase contrast light microscope after 7 days of culture.

Table I

Dose-dependent H7 activity on the human neuroblastoma cell variants

Cell line	H7 (μM)			
	3.5	7	14	28
SK-N-BE(2)	−*	−	+/−	+
LAN-1	−	+	+	++
LAN-5	ND	ND	++	++

* Neurite outgrowth: −, no neurite formation; +/−, appreciable neurite outgrowth; +, ex-novo neurite formation protruding from several cells; ++, evident neurite outgrowth from many cells forming neurite network; and ND, not detected

like processes. This morphological type contains the neurofilaments [8] and appears related to neuronal cell with properties of noradrenergic neurons. I-type cell is morphologically intermediate between two cell types: N, described above and epithelial-like (S) cells with properties of melanocyte and Schwann cells, and containing the intermediate filament-related protein vimentin [9,10].

Our results show that H7 induces neuritogenesis in human neuroblastoma cells as well as in murine cells. Since H7 is a specific inhibitor of PKC, it appears that inhibition of PKC activity is involved in the morphological differentiation of the neuroblast.

Minana et al. [5], treating the neuro-2a cells with 85 and 500 μM H7 observed over 60% of the cells with neurites. We detected morphological differentiation at concentrations of H7 ranging between 7 and 28 μM showing that H7 is more effective on human cells.

Taken as a whole, the results indicate that PKC is involved in morphological differentiation and neurite outgrowth of both murine and human neuroblastoma cells. In our hands, H7 induced cell differentiation in both neuroblast and intermediate cell types.

Since N- and I-type cell variants are thought to be associated at distinct stages of neuronal development [11], H7 appears generally active on neuroblastoma cells having different morphological and biochemical characteristics.

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