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Inhibition of protein kinase C arrests proliferation of human tumors

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We have shown that inhibition of protein kinase C by 1-5-isoquinolinylsulfonyl-2-methylpiperazine, H7, induces differentiation and inhibits proliferation of Neuro 2a cells. We have now tested if H7 is able to inhibit proliferation of: 1) human tumor cell lines from tissues other than brain; and 2) primary cultured cells from several human brain tumors. H7 inhibits, in a dose-dependent manner, proliferation of all human tumor cell lines tested and of primary cultured cells from human brain tumors. These results indicate that inhibition of protein kinase C inhibits proliferation of tumoral cells, therefore, H7, and likely other inhibitors of protein kinase C, could be useful in the clinical treatment of brain (and probably other) tumors.

Tumor proliferation; Protein kinase C; H7; Brain tumor

1. INTRODUCTION

Tumors originate from cells with altered growth regulation which proliferate exponentially. Much recent research on the clinical treatment of tumors has been focused on the use of agents that induce differentiation and thereby inhibit proliferation [1,2].

We have recently found that inhibition of protein kinase C, the Ca^{2+}/phospholipid-dependent protein kinase, induces differentiation and inhibits proliferation of the clonal cell line Neuro-2a, C-1300 mouse neuroblastoma [3,4]. The aim of the present work was to assess if the inhibition of protein kinase C could also inhibit proliferation of: (i) human tumor cell lines from tissues other than brain; and (ii) human brain tumors in culture.

2. MATERIALS AND METHODS

2.1. *Cell lines*

The human rhabdomyosarcoma A-204, osteogenic sarcoma HOS and breast adenocarcinoma MCF-7 cell lines were kindly provided by Dr Llombart (Dept. Pathology, University of Valencia, Spain). The cells were cultured in RPMI-1640 medium supplemented with 20% fetal bovine serum, 2 mM glutamine, 100 IU/ml of penicillin and 100 μg/ml of streptomycin (Flow Laboratories) at 37°C in 5% CO_2 in a humidified incubator. For cell viability determination, cells were seeded at 200000/ml, after 24 h, H7 was added and, 24 h later, cells were harvested by trypsinization. Viability was determined by the Trypan blue exclusion method. For assays of DNA synthesis, cells were seeded at 100000/ml in Nunc plates. H7 was added after 24 h and 3 h later 5 μCi of [^{3}H]thymidine (5 Ci/mmol; 1 mCi/ml) were added. Cells were incubated for one hour and trichloroacetic acid precipitable radioactivity was counted.

2.2. *Human brain tumors*

Cells derived from the indicated human brain tumors were cultured in Ham's F10 medium containing 10% inactivated (56°C, 30 min) fetal bovine serum and (per ml) 100 IU penicillin, 100 μg streptomycin and 90 μg gentamicin. Medium was changed at least twice weekly and no passage was carried out to avoid alterations, i.e. in chromosome number. After 8–20 days of culture H7 was added to some flasks. One hour later 40 μCi/ml of [^{3}H]thymidine (25 Ci/mmol) were added and, after 20 h of incubation, the radioactivity incorporated into trichloroacetic acid-precipitable material was counted.

3. RESULTS AND DISCUSSION

As shown in Table I, H7 inhibits the proliferation of all human tumor cell lines tested. The inhibition ranged between 33% for breast adenocarcinoma and 75% for rhabdomyosarcoma. H7 did not affect cellular viability

Table I

Effect of H7 on DNA synthesis and cell viability of three human tumor cell lines

Cell line	DNA synthesis (% of control)	Cell viability (%)
Osteogenic sarcoma (HOS)	51	39
Breast adenocarcinoma (MCF-7)	67	93
Rhabdomyosarcoma (A-204)	25	89

Cells were cultured in the absence or the presence of 500 μM H7 for 3 h (DNA synthesis) or 24 h (cell viability). Viability for non-treated cells ranged between 90 and 100%.

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Table II

Effect of H7 on the proliferation of human brain tumors

Tumor	% of inhibition of DNA synthesis/mg protein	
	500 μM H7	700 μM H7
Multiform glioblastoma	46	–
Hypophyseal adenoma	77	94
Meningioma 1	87	88
Meningioma 2	80	87
Meningioma 3	67	87
Metastatic carcinoma	76	89

Cells derived from the indicated human brain tumors were cultured as described in Materials and Methods. Incorporation of [^{3}H]thymidine into trichloroacetic acid-precipitable material was determined in the absence or the presence of the indicated concentrations of H7.

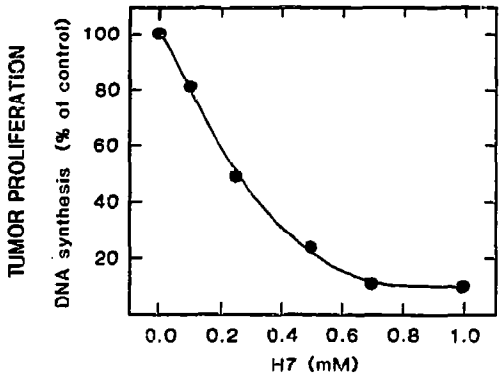

Fig. 2. Dose-dependent inhibition by H7 of DNA synthesis in cells from a metastatic carcinoma. Cells were incubated in the absence or the presence of the indicated amounts of H7 and DNA synthesis was measured as described in Table I. Values are expressed as % of control.

of breast adenocarcinoma or rhabdomyosarcoma but was significantly cytotoxic for osteogenic sarcoma.

Primary cultured cells were prepared from the following tumors: multiform glioblastoma, hypophyseal adenoma, meningioma and metastatic carcinoma.

As shown in Fig. 1, 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H7) induces the extension of neurite-like processes in primary cultured cells from hypophyseal adenoma and multiform glioblastoma. Similar results were obtained with the other tumors tested.

As shown in Table II, H7 markedly inhibits the proliferation of all human brain tumors tested. At 500 μM H7, the inhibition ranged between 46% for the

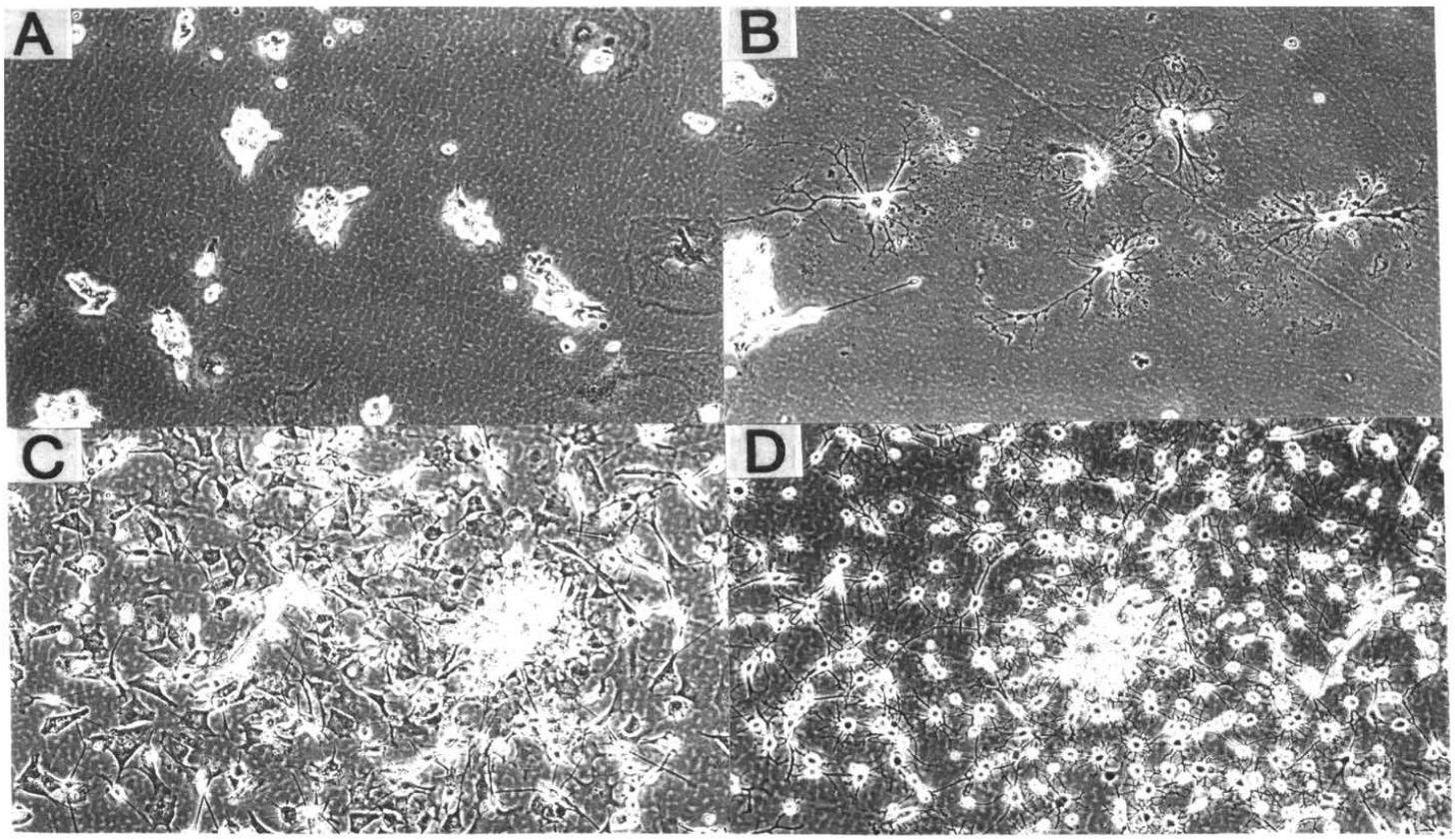

Fig. 1. Effect of H7 on the morphology of primary cultured human brain tumor cells. Cells were incubated with (B,D) or without (A,C) 500 μM H7 and photographed after 15 h of treatment. (A) and (B) show cells from hypophyseal adenoma and (C) and (D) from multiform glioblastoma.

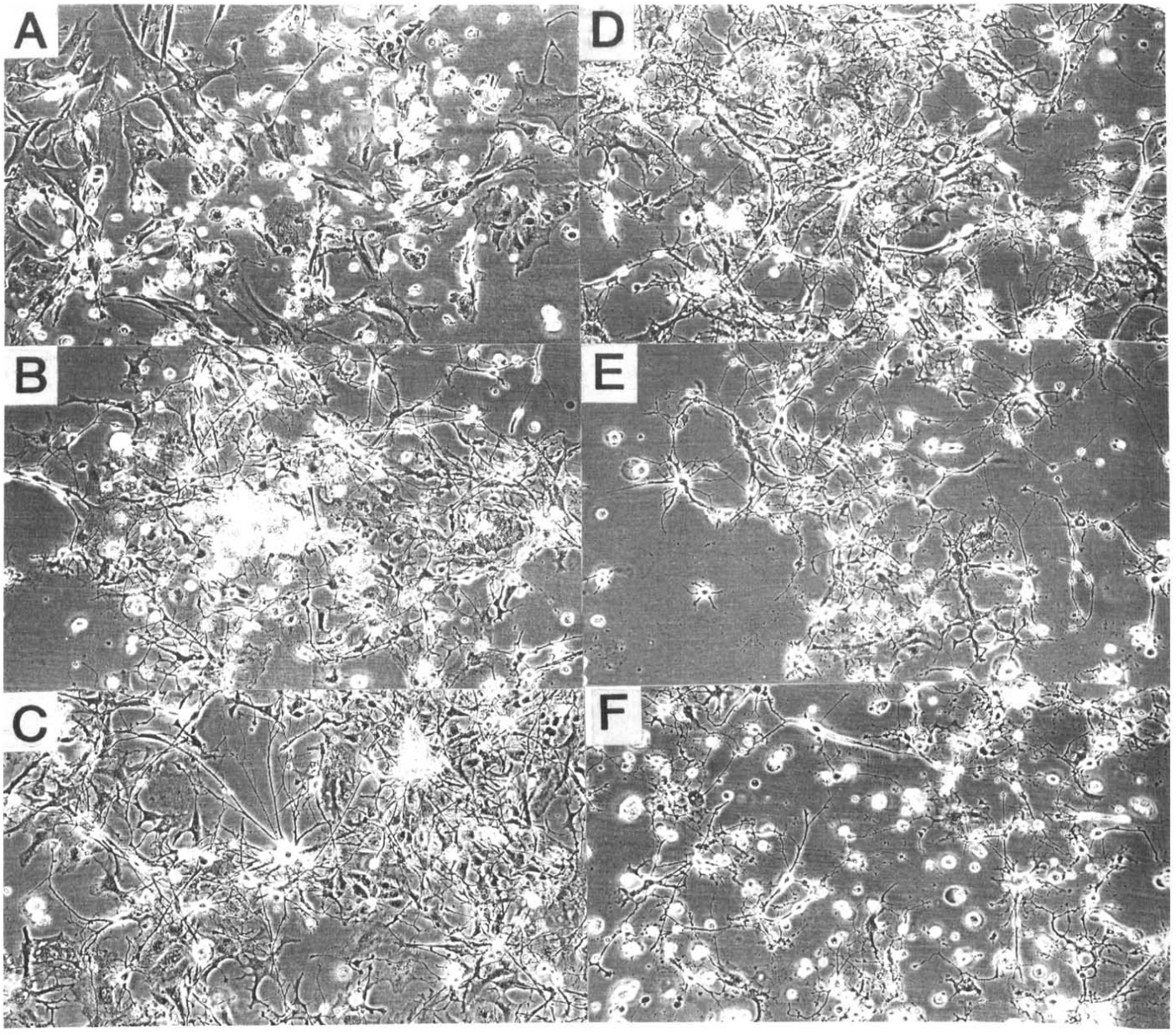

Fig. 3. Effect of different concentrations of H7 on the morphology of cells from a metastatic carcinoma. Cells were incubated in the absence (A) or the presence of 0.1 (B), 0.25 (C), 0.5 (D), 0.7 (E) or 1.0 (F) mM H7. Cells were photographed 15 h after the addition of H7.

glioblastoma and 87% for the meningioma. At 700 μM H7 the proliferation of all tumors tested was inhibited by at least 87%. It should be noted that H7, at these doses, did not significantly affect cell viability.

The percentage of inhibition was dependent on the concentration of H7 added. This is shown in Fig. 2 for the metastatic carcinoma. The effect was maximum (89% inhibition) at 700 μM H7. The morphological changes induced by increasing concentrations of H7 are shown in Fig. 3. These results indicate that H7, and possibly other inhibitors of protein kinase C, could be useful clinically.

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