Effect of Butyrate and Corticosteroids on Retinoblastoma In Vitro and In Vivo

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The effect of sodium butyrate (NaB) alone and in combination with a glucocorticoid was studied on Y-79 retinoblastoma cells both in vitro and in vivo. Both NaB (0.5 and 4 mM) and hydrocortisone (1–100 μM) added separately to cells grown in suspension resulted in a small growth inhibition (<15%). A marked synergistic effect (75–77% growth inhibition) was observed in vitro when NaB and hydrocortisone were added in combination at all the concentrations tested. With in vivo experiments using the nude mouse model of retinoblastoma, the combination of NaB and methylprednisolone did not inhibit tumor growth. Invest Ophthalmol Vis Sci 32:1711–1713, 1991

In this investigation, the ability of sodium butyrate (NaB) and glucocorticoids to inhibit retinoblastoma growth in vitro and in vivo was studied. In previous in vitro studies of Y-79 cells growing in attachment culture, it was found that NaB induces growth inhibition and morphologic changes including an elongated appearance of cells and retraction of long processes. These changes were interpreted to indicate that NaB causes cellular differentiation. We investigated growth inhibition and morphologic changes in suspension cultures of Y-79 cells by NaB and glucocorticoids and determined whether these agents cause growth inhibition or differentiation of retinoblastoma in an in vivo model.

Materials and Methods

In Vitro

The Y-79 retinoblastoma cells were maintained in suspension culture in RPMI medium containing 10% fetal bovine serum (FBS). The cells were conditioned for 1 week in modified Eagle’s medium (MEM, GIBCO, Grand Island, NY) with 10% FBS, 2 mM glutamine, 100 units/ml of penicillin, and 100 units/ml of streptomycin for 1 week before the experiment. Conditioned cells were counted using a hemocytometer, and 2 × 10⁶ cells were seeded in 35-mm tissue culture plastic dishes. The NaB and steroids alone and in combination were added on the third day of culture with the change of MEM medium. The Y-79 cells were grown under 12 conditions: (1) 0.5 mM NaB, (2) 4 mM NaB, (3) 0.001 mM hydrocortisone sodium succinate (HC), (4) 0.01 mM HC, (5) 0.1 mM HC, (6) 0.5 mM NaB and 0.001 mM HC, (7) 0.5 mM NaB and 0.01 mM HC, (8) 0.5 mM NaB and 0.1 mM HC, (9) 4 mM NaB and 0.001 mM HC, (10) 4 mM NaB and 0.01 mM HC, (11) 4 mM NaB and 0.1 mM HC, and (12) no agent (control). Each condition was tested with three identical cultures. After a 48-hr incubation at 37°C, three samples from each culture were assayed for cell viability by trypan blue staining.

In Vivo

Athymic “nude” (nu/nu) mice from the Charles River Laboratories (Wilmington, MA) each received a dorsal subcutaneous injection of 1 × 10⁷ retinoblastoma cells. The tumor cells were derived from Y-79 cells subcutaneously passaged in athymic mice. The mice were housed in a laminar flow room and fed with sterile water and rodent lab chow from Purina (Richmond, IN). All mice were examined daily for tumors, which (when visible) were measured with calipers daily. Control mice received no treatment. The 24 experimental mice received a subcutaneous depot injection of 20 mg/kg of methylprednisolone (MP) (Upjohn, Kalamazoo, MI), equivalent to 100 mg/kg
of HC. Steroid treatment was administered 1 day before tumor cell transplantation and again when the tumors were initially visible (days 6–73), as described previously. Thereafter the experimental animals received daily intraperitoneal injections of NaB (Sigma, St. Louis, MO) at a dose of 1.6 g/kg, corresponding to 75% of the lethal dose for 10% of the animals (unpublished data).

Of the 24 control mice injected with retinoblastoma cells, 7 had tumors; of the 24 experimental mice injected with retinoblastoma cells, 13 had tumors. Of the controls, one mouse was killed after 5 days, three after 10 days, and three after 18 days. Of the experimental mice, three were killed after 5 days, three after 6 days, one after 10 days, one after 14 days, one after 15 days, and three after 18 days. The mice were necropsied, and the tumors were excised, measured, and fixed in 10% phosphate-buffered formalin. The tumor size was calculated in terms of the geometric mean diameter (length × width × height). Statistical analysis of the tumor sizes was done.

**Results**

**In Vitro**

After a 48-hr incubation with NaB and HC, the growth of Y-79 cells was inhibited with respect to untreated controls. The most marked inhibition was a result of incubation with 0.5 mM NaB and 0.1 mM HC (77%) and with 4 mM NaB and 0.1 mM HC (75%; Fig. 1). Significant inhibition was also noted after treatment with 4 mM NaB and 0.01 mM HC (72%). Less inhibition was noted after incubation with 4 mM NaB and 0.001 mM HC (63%), with 0.5 mM NaB and 0.01 mM HC (60%), and with 0.5 mM NaB and 0.001 mM HC (46%). Finally, minimal inhibition was recorded after treatment with either NaB or HC alone. No cell differentiation was noted during examination with inverted microscopy.

**In Vivo**

When the tumor size was compared between control mice and experimental mice, no statistically significant difference was noted for any given period (Fig. 2). The histologic sections of tumors from mice treated with NaB and HC showed no signs of differentiation. When observed in a masked fashion, using criteria previously described, no difference between control and experimental sections was noted.

**Discussion**

The growth inhibition of retinoblastoma has been investigated experimentally with both in vivo and in vitro systems. Growth of both human retinoblastoma xenografts in athymic mice and Y-79 retinoblastoma cells in suspension culture is inhibited by 1,25(OH)2 vitamin D3. However, significant systemic toxicity resulted, and therefore nontoxic alternatives would be valuable. One advantage of NaB is its low toxicity in vivo.

When Y-79 cells were grown in suspension with 0.5 mM or 4 mM NaB and 0.1 mM HC for 48 hr, 77% and 75% growth inhibition resulted. There was only 16% inhibition with 4 mM NaB alone, and 0.1 mM HC alone caused only 9% inhibition. There appears to be a synergistic effect in vitro, causing inhibition by NaB and HC to be far greater than inhibition by either agent alone.
Treatment of Y-79 cells with NaB causes cell growth inhibition, cell differentiation, and cell death. These cytopathic changes may be due to the agent's molecular action. It has been shown in vitro that NaB alters gene expression and causes translocation from the cytoplasm to the nucleus of the regulatory subunit of type II cyclic adenosine monophosphate-dependent protein kinase in Y-79 cells.

The dramatic inhibition of retinoblastoma growth in vitro led us to test the combination of NaB and MP in vivo to see if there was an inhibition of xenografts in athymic mice. In murine carcinoma and sarcoma tumors at 200 mg/kg and in murine lymphosarcoma tumors at 100 mg/kg, HC has been shown to inhibit tumor growth. A concentration of MP equivalent to 100 mg/kg of HC was used. Xenografted retinoblastoma tumors in mice treated with NaB and MP showed no significant difference in tumor size or histologic features compared with controls. This discrepancy between in vitro and in vivo growth inhibition may be due to a low concentration of the agents at the tumor site in vivo. These concentrations may be inadequate to cause growth inhibition or differentiation. The metabolic degradation of the agents in vivo may contribute to this effect.

Although HC has been reported to inhibit tumor growth of murine sarcomas and carcinomas, it also can cause immunosuppression of natural killer cells in vitro. Natural killer cells can destroy tumor cells in vitro and in vivo. The higher number of tumors in mice exposed to HC compared with controls may be a result of a decreased number of natural killer cells. The present experiments indicate that the combination of NaB and HC does not inhibit tumor growth in vivo.

Key words: butyrate, glucocorticoid, retinoblastoma

References