8-Cl-Adenosine Induces Growth Arrest without Differentiation of Primary Mouse Epidermal Keratinocytes

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In some cell systems, the antiproliferative effects of 8-Cl-cAMP, a site-selective cAMP analog specific for the type 1 cAMP-dependent protein kinase, are mediated by its metabolite, 8-Cl-adenosine. These effects were once thought to be specific to transformed cells. We investigated the ability of 8-Cl-adenosine to regulate growth and differentiation in primary cultures of mouse epidermal keratinocytes. A 24 h exposure of keratinocytes to 8-Cl-adenosine inhibited [3H]thymidine incorporation in a dose-dependent manner with an apparent IC₅₀ of 7.5 µM, and these effects were completely reversible. To determine the ability of 8-Cl-adenosine to induce differentiation of primary keratinocytes, we measured keratin-1 expression and transglutaminase activity, markers of early and later stages of keratinocyte differentiation, respectively. Interestingly, exposure of keratinocytes to 25 µM 8-Cl-adenosine for 24 h had no effect on keratin-1 expression or transglutaminase activity. The 8-Cl-adenosine-induced growth arrest of keratinocytes required uptake of the compound and was accompanied by an increase in protein expression of the cyclin-dependent protein kinase inhibitor p21WAF1/Cip1. These results demonstrate that 8-Cl-adenosine inhibits growth in a nontransformed/non-immortalized cell system, possibly through an elevation in p21WAF1/Cip1 protein levels, without inducing differentiation.

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Recently, much attention has been focused on the use of nontoxic biologic agents that may potentially restore regulatory control to cellular proliferation in neoplastic cells. One such biologic agent, 8-Cl-cAMP, has gained considerable attention due to its potent antiproliferative properties against transformed cells. An initial phase I clinical trial using 8-Cl-cAMP as an antineoplastic drug has been performed (Tortora et al, 1995). Important, though, are the observations that in some cells the antiproliferative effects of 8-Cl-cAMP are mediated by its metabolite, 8-Cl-adenosine. For example, the 8-Cl-cAMP-induced inhibition of proliferation and downregulation in mdr1 expression in the breast cancer cell line MCF7 is blocked if adenosine deaminase activity, which converts adenosine to inosine, is elevated in the culture medium (Scala et al, 1995). Also, 8-Cl-cAMP-mediated inhibition of proliferation in CHO cells is almost completely blocked when the cells are cultured in either serum-free conditions or medium containing heat-inactivated serum (Van Lookeren Campagne et al, 1991), conditions that reduce or eliminate phosphodiesterase activity and inhibit the conversion of 8-Cl-cAMP to 8-Cl-adenosine. Along these same lines, use of a phosphodiesterase-resistant form of 8-Cl-cAMP, Sp-8-Cl-cAMP, but not the parental compound, failed to inhibit proliferation in the colorectal cancer cell line LS174T (Carlson et al, 2001). And finally, 8-Cl-cAMP-induced inhibition of proliferation of nontransformed fibroblast, myoblast, and endothelial cells is dependent upon its conversion to 8-Cl-adenosine (Han et al, 1993). Thus, in some cell systems, the antiproliferative effects of 8-Cl-cAMP apparently are mediated by 8-Cl-adenosine. In addition, it was once thought that the antiproliferative effects of this nucleoside were specific to transformed cells; however, 8-Cl-adenosine inhibits proliferation in normal fibroblast, myoblast, and endothelial cells (Han et al, 1993), as well as in both nontransformed and c-ras-transformed 3T3 cells (Noguchi et al, 1998).

Primary epidermal keratinocytes represent a nontransformed cell type that proliferates as part of its normal function. Specifically, these cells continuously divide in vivo to regenerate epidermal cells lost to the environment. Keratinocytes also differentiate in culture in response to several agents (e.g., gangliosides G₅₁b and G₃₂₁b, elevated medium Ca²⁺ levels, and 1,25-dihydroxyvitamin D₃). These agents can induce markers of both early (e.g., keratin-1 and keratin-10 expression) and late (e.g., transglutaminase activity) differentiation (Yada et al, 1991; Paller et al, 1995; and reviewed in Bikle and Pillai, 1993). In general, agents that induce keratinocyte growth arrest also trigger the complete program of differentiation; however, these two processes can be dissociated in the case of some keratinocyte regulators. For example, transforming growth factor β (TGF-β) induces growth arrest without an accompanying increase in the expression of keratinocyte differentiation markers (Bikle and Pillai, 1993; Hu et al, 1998); however, the mechanism of this dissociation is unknown.

As discussed above, in many cell types the antiproliferative effects of 8-Cl-cAMP are mediated by its metabolite, 8-Cl-adenosine. In
addition, adenosine and its related adenine nucleotides (ATP, ADP, AMP) inhibit proliferation in normal human keratinocytes (Cook et al., 1995; Brown et al., 2000). The mechanism by which this occurs is unknown at this time, however. In light of these studies, we sought to determine if 8-Cl-adenosine exerted any antiproliferative effects against a nontransformed, nonimmortalized cell system, primary mouse keratinocytes. We found that 8-Cl-adenosine triggered reversible growth arrest of primary mouse epidermal keratinocytes without inducing an increase in markers of early or late differentiation. Thus, this compound appears to act in a similar manner to TGF-β but is a small nonpeptide molecule capable of inhibiting the proliferation of keratinocytes without affecting their differentiation status.

MATERIALS AND METHODS

Materials 8-Cl-adenosine was purchased from Biolog (La Jolla, CA). Anti-p21WAF1/CIP1 (Ab-4), Oncogene Sciences (Cambridge, MA), and anti-p21WAF1/CIP1 (13436E6), Pharmlingen (San Diego, CA), were used for this study. p21WAF1/CIP1 was purchased from Covance Research, formerly Berkeley Antibody (Richmond, CA). Goat antikeratin antibody (1:1000) was purchased from Promega (Madison, WI). SuperSignal Chemiluminescent substrate detection kits were obtained from Pierce (Rockford, IL). Nitrocellulose was purchased from Fisher Scientific. [3H]Thymidine, [3H]putrescine and [125I]-labeled goat antirabbit IgG were purchased from Du Pont/NEN (Boston, MA). Propidium iodide was purchased from Molecular Probes (Eugene, OR). All other reagents were obtained from standard suppliers and were of the highest grade available.

Cell culture Primary epidermal keratinocytes were prepared from 1- to 3-day old neonatal ICR mice according to the method of Marcello et al. (1978). Briefly, after trypsinization of the skin, the epidermis was mechanically separated from the dermis and epidermal cells were released by scraping. These cells were then collected by centrifugation and plated in six-well dishes in keratinocyte medium consisting of minimum essential medium containing 25 μM Ca2+, 2% (vol/vol) dextral fetal bovine serum (FBS), 2 mM glutamine, 5 ng per ml epidermal growth factor, ITS (6.25 μg per ml insulin, 6.25 μg per ml transferrin, 6.25 ng per ml selenious acid + 0.1% (vol/vol) penicillin, 100 μg per ml streptomycin, and 0.25 μg per ml fungazone. After an overnight incubation, the cells were refed with serum-free keratinocyte medium (SFKM), in which 2% (vol/vol) dextral FBS was replaced with 90 μg per ml bovine pituitary extract. Cells were refed with fresh medium every 1–3 d.

Measurement of DNA synthesis For measurement of [3H]thymidine incorporation, near-confluent cultures were refed with SFKM containing various concentrations of 8-Cl-adenosine. After the times indicated in the figure legends, cells were labeled with 1 μCi per ml [3H]thymidine for an additional hour in the continued presence of 8-Cl-adenosine. Cultures were washed twice with phosphate-buffered saline without calcium or magnesium (PBS) and reactions were terminated using ice-cold 5% trichloroacetic acid (TCA). Cells were washed with an additional volume of 5% TCA and with distilled water, and were solubilized in 0.3 M NaOH for 10 min at 50°C. The amount of [3H]thymidine incorporated into DNA was determined by liquid scintillation counting.

Flow cytometric analysis Un synchronized near-confluent cultures of keratinocytes were refed with SFKM containing the indicated agents. After 24 h, the media as well as one 1 × PBS wash (to obtain floating cells) were collected. The attached cells were harvested and pooled with the cells that had become detached. In some experiments the media and the PBS’ wash were aspirated and only the attached cells were collected and processed for flow cytometric analysis. The combined cell pellet was washed once with ice-cold PBS, gently resuspended in ice-cold 70% EtOH, and stored at 4°C until processing. DNA content of the nuclei was determined by staining nuclear DNA with propidium iodide (50 μg per ml, 30 min) and measuring the relative DNA content of nuclei of 10,000 events, using a Becton Dickinson FACSCalibur fluorescence-activated cell sorter. MODFIT DNA and CELLQUEST analysis software were utilized to determine the proportion of nuclei in each phase of the cell cycle.

RESULTS

8-Cl-adenosine inhibits proliferation in primary mouse keratinocytes in a dose- and time-dependent manner In this study we sought to determine whether 8-Cl-adenosine, an agent known to induce growth arrest and/or differentiation in transformed cells (Carlson et al., 2000; 2001) and nontransformed cells (Han et al., 1993), could elicit growth arrest and/or differentiation in a nontransformed/nonimmortalized primary cell, whose principal function depends in part upon continuous proliferation. A 24 h exposure of keratinocytes to 8-Cl-adenosine inhibited [3H]thymidine incorporation in a dose-dependent manner with an apparent IC50 of 7.5 μM (Fig 1A). 8-Cl-adenosine inhibited growth of primary mouse keratinocytes within 1 h of treatment with a 70%–80% inhibition of [3H]thymidine incorporation observed after 24 h (Fig 1B). Maximal inhibition was observed between 18 and 24 h (Fig 1B).

The growth inhibitory effects of 8-Cl-adenosine are completely reversible Previous studies have demonstrated that 8-Cl-adenosine exerts an irreversible inhibition of proliferation in colorectal cancer cells (Tagliaferri et al., 1988). We sought to determine if the antiproliferative effects we observed in primary mouse keratinocytes exhibited a similar phenomenon. Treatment of keratinocytes with 8-Cl-adenosine for either 24 or 48 h followed by a mitotic removal and replenishment of fresh medium resulted in a reversible growth inhibition of these cells (Fig 2). Not only did these cells reestablish their proliferative capabilities but the cells that had been treated with 8-Cl-adenosine...
for 24 h exhibited growth rates greater than those cells that had not been previously exposed to 8-Cl-adenosine.

8-Cl-adenosine induces a G0/G1 cell cycle arrest We utilized flow cytometric analysis to address changes in cell cycle profile relative to cellular growth/proliferation in primary mouse keratinocytes. In pooled attached and detached cells, 8-Cl-adenosine induced a significant increase in the population of keratinocytes in G0/G1 concomitant with a decrease in the number of cells in S phase (Table I). These results were similar to those observed in keratinocytes treated with TGF-β, a known inducer of G0/G1 arrest in a number of cell systems (Hu et al., 1998), including keratinocytes (Alexandrow and Moses, 1995). A small but statistically significant increase in the proportion of cells in G2/M was also detected in 8-Cl-adenosine-treated keratinocytes. On the other hand, there was no increase in the number of cells in the sub-G0/G1 phase, generally considered to be indicative of apoptotic cells. In additional experiments, we examined the effect of 8-Cl-adenosine on attached cells only. Again, 8-Cl-adenosine decreased the proportion of cells in S phase and increased the percentage of cells in G0/G1 (Table II). These results were similar to the effects observed when the attached and detached cells were pooled and analyzed (Table I) and were also once more similar to the effects of TGF-β.

8-Cl-adenosine does not cause keratinocyte differentiation Numerous agents that elicit growth arrest of keratinocytes also trigger their differentiation. Therefore, we sought to determine if 8-Cl-adenosine induced a differentiated phenotype in keratinocytes. We analyzed 8-Cl-adenosine-treated keratinocytes for either keratin-1 expression or transglutaminase activity, early and later differentiation markers, respectively. Keratinocytes were treated for 18 or 24 h with 8-Cl-adenosine and analyzed for keratin-1 expression by Western blot analysis. Figure 3(A) illustrates representative Western blots demonstrating that 8-Cl-adenosine did not increase expression of this differentiation marker. On the other hand, the positive controls, elevated extracellular Ca2+ levels (125 μM) and 1,25-dihydroxyvitamin D3 (10 nM), both inducers of differentiation in keratinocytes (reviewed in Bilde and Pillai, 1993), increased keratin-1 protein expression (Fig 3A). PhosphorImager densitometric analysis revealed that Ca2+ and 1,25-dihydroxyvitamin D3 increased keratin-1 protein expression from 2- to 8-fold over control-treated keratinocytes (Fig 3B). 8-Cl-adenosine had no effect on keratin-1 protein expression. In addition, a 24 h exposure to 8-Cl-adenosine failed to alter transglutaminase activity, a marker of late-stage differentiation, at concentrations of either 10 or 25 μM. On the other hand, the positive control, elevated extracellular Ca2+ levels (1 mM), increased transglutaminase activity 8-fold over that observed in control-treated keratinocytes (Fig 4). A longer (48 h) exposure to

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Figure 1. 8-Cl-adenosine inhibits growth in a dose- and time-dependent manner. Primary mouse keratinocytes were treated with the indicated concentrations of 8-Cl-adenosine for 24 h (a) or with 10 μM (◇) or 25 μM (□) 8-Cl-adenosine for the indicated times (b), and [3H]thymidine incorporation was determined as indicated in Materials and Methods. Data represent the mean ± SEM of seven (a) and four (b) experiments. (a) *p < 0.01; (b) all values significantly different from control-treated cells, p < 0.01.

Figure 2. The 8-Cl-adenosine-induced inhibition of proliferation is completely reversible. Primary mouse keratinocytes were treated with 8-Cl-adenosine (8-Cl-ad) (10 or 25 μM) or control vehicle (H2O) for 24 (hatched bars) or 48 (open bar) h. The cells were washed twice with PBS and once with SFKM prior to the addition of fresh SFKM ± 8-Cl-ad (10 or 25 μM). The cells were incubated for an additional 24 h prior to measuring the incorporation of [3H]thymidine (Materials and Methods). Data represent the mean ± SEM of four experiments; *p < 0.01 compared to control-treated cells.
8-Cl-adenosine also failed to increase transglutaminase activity (0.65-fold ± 0.08-fold over control), although again the positive control of elevated extracellular Ca\(^{2+}\) levels (1 mM) stimulated activity (6.5-fold ± 1.5-fold over control, \(n = 3\); \(p < 0.001\)).

**Potential mechanisms of 8-Cl-adenosine-induced growth arrest** Recent data from the laboratory of Dr. Paul Cook has indicated that adenosine analogs can inhibit keratinocyte growth by a mechanism that requires uptake of the compounds into the cell (Brown et al., 2000). To determine if a similar requirement for uptake of 8-Cl-adenosine exists, we examined the effect of the nucleoside transport inhibitor nitrobenzylthioinosine (NBTI) on 8-Cl-adenosine-induced growth arrest by flow cytometric analysis. We have previously shown that 100 \(\mu\)M NBTI inhibits more than 90% of the uptake of the nucleoside thymidine into keratinocytes (Griner and Bollag, 2000). Preventing uptake of 8-Cl-adenosine into keratinocytes with NBTI blocked the ability of 8-Cl-adenosine to reduce the proportion of cells in the S phase and increase the percentage in \(G_0/G_1\) (Table III). Thus, our results indicate that uptake of 8-Cl-adenosine into the cells is necessary for the compound to elicit growth arrest.

Regulation of \(G_0/G_1\) cell cycle arrest has been attributed to a number of cellular proteins, including p33, the CDK inhibitors (p21\(^{WAF_1/CIP_1}\), p27\(^{KIP_1}\), and p57\(^{KIP_2}\)), and the specific inhibitors of CDK4 (p16\(^{INK_4A}\), p15\(^{INK_4B}\), p18\(^{INK_4C}\), and p19\(^{INK_4D}\)), known as the INK4 proteins (Sherr, 1996). We investigated the effect of 8-Cl-adenosine on p21\(^{WAF_1/CIP_1}\), because of its recognized importance to keratinocyte growth arrest (Missero et al., 1995, 1996; Di Cunto et al., 1998). Western blot analysis of 8-Cl-adenosine-treated primary mouse keratinocytes revealed an increase in the levels of p21\(^{WAF_1/CIP_1}\) protein above control-treated cultures at as early as 6 h of treatment (data not shown) with a pronounced 2- to 3-fold increase observed by 16–24 h of exposure (Fig 5).

**DISCUSSION**

It has become increasingly clear that the use of 8-Cl-cAMP or 8-Cl-adenosine for the control of cellular proliferation is not as simple as once believed. It now appears that this cAMP analog and its active metabolite exert differential effects on a wide variety of cells. In some cases, the antiproliferative effects of 8-Cl-cAMP are mediated by 8-Cl-adenosine (Van Lookeren Campagne et al., 1991; Scala et al., 1995), whereas in some cell systems 8-Cl-cAMP inhibits growth independent of its conversion to 8-Cl-adenosine (Noguchi et al., 1998). In any event, the signal transduction mechanisms by which either of these two agents induce their antiproliferative effects has not been clearly defined. We have sought to determine the effect of 8-Cl-adenosine on the growth of a nontransformed/ nonimmortalized cell system, primary cultures of mouse keratinocytes. We chose this cell system because in \textit{vivo} keratinocytes must proliferate as part of their normal function; thus these cells represent a good model for investigating antiproliferative effects in a normal cell type.

8-Cl-adenosine inhibited growth in primary cultures of mouse keratinocytes to a dose- and time-dependent manner (Fig 1). Interestingly, the effects of 8-Cl-adenosine on keratinocyte growth were completely reversible (Fig 2). These findings are in contrast to the irreversible growth inhibitory effects observed in the
results in a decline in the protein levels of p21 WAF1/Cip1. Elevation Dotto and colleagues, however, reported that p21 WAF1/Cip1—
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and its subsequent removal resulted in enhanced [3H]thymidine 
other hand, overexpression of p21 WAF1/Cip1 was also found to 
significantly (but does not cease) once the cells attain confluence. 
probably due to the fact that keratinocyte proliferation decreases 
transport inhibitor NBTI suggest that uptake of the compound into 
Colorectal cancer cell line LS174T (Tagliaferri et al, 1988) and 
suggest the potential for differences in the mechanism by which this 
nucleoside inhibits growth in nontransformed versus transformed 
cells. Along these lines, 8-Cl-adenosine induces apoptosis in several 
colorectal cancer cell lines (Carlson et al, 2000), whereas we did not 
detect any apoptosis in 8-Cl-adenosine-treated keratinocytes (Table I). Interestingly, treatment with 8-Cl-adenosine for 24 h 
and its subsequent removal resulted in enhanced [3H]thymidine 
incorporation relative to control cultures. This “rebound” effect is 
probably due to the fact that keratinocyte proliferation decreases 
significantly (but does not cease) once the cells attain confluence. 
Thus, if the cells are prevented from becoming completely 
confluent by 8-Cl-adenosine treatment, removal of the 8-Cl-
adenosine-induced inhibition will lead to enhancement of DNA 
synthesis relative to the confluence-inhibited control cultures.
Flow cytometric analysis revealed that 8-Cl-adenosine induced a 
G0/G1 arrest. This cell cycle arrest was associated with elevations in 
the cyclin-dependent kinase inhibitor p21 WAF1/Cip1 (Fig 5). We do 
not know at this time if removal of 8-Cl-adenosine, a condition 
that releases keratinocytes from their apparent cell cycle arrest, 
results in a decline in the protein levels of p21 WAF1/Cip1. Elevation 
in p21 WAF1/Cip1 protein is associated with differentiation in a 
number of cell systems (Jiang et al, 1994; Datto et al, 1995; Missero et al, 1995). 8-Cl-adenosine-induced elevations in p21 WAF1/Cip1 
protein, however, were not accompanied by keratinocyte differ-
entiation, as determined by changes in keratin-1 expression or 
transglutaminase activity between 18 and 48 h of treatment 
(Figs 3, 4). These results are similar to that observed with 
TGFB (an inducer of p21 WAF1/Cip1) in that G0/G1 arrest occurs 
without a concomitant induction of differentiation (Hu et al, 1998). 
Dotto and colleagues, however, reported that p21 WAF1/Cip1—
knockout mouse keratinocytes exhibited a downregulation in the 
late-stage keratinocyte differentiation markers loricrin and involu-
crin (Missero et al, 1996), suggesting that p21 WAF1/Cip1 is 
required for this stage of differentiation in keratinocytes. This effect was not 
observed in keratinocytes devoid of the closely related cyclin-
dependent kinase inhibitor p27Kip1 (Missero et al, 1996). On the 
other hand, overexpression of p21 WAF1/Cip1 was also found to 
prevent differentiation marker expression (Di Cunto et al, 1998).
Thus, the exact role of p21 WAF1/Cip1 in the control of keratinocyte 
differentiation has yet to be clarified.
The mechanism through which 8-Cl-adenosine increases 
p21 WAF1/Cip1 expression and exerts its growth inhibitory effect is 
not clear. In studies in progress we have observed an 8-Cl-
adenosine-elicited increase in keratinocyte p53 protein levels by 
approximately 4 h (data not shown). Similarly, in colorectal cancer 
cells both p53 and p21 WAF1/Cip1 levels increase upon treatment 
with 8-Cl-adenosine (Carlson et al, 2001). p21 WAF1/Cip1 levels also 
are elevated in p53 mutant cells, however, (Dransfield et al, 
unpublished observations), suggesting perhaps both p53-dependent 
and—independent mechanisms for the 8-Cl-adenosine-induced rise 
in p21 WAF1/Cip1 levels. In addition, results with the nucleoside 
transport inhibitor NBTI suggest that uptake of the compound into 
the cells is required. This finding is similar to the data of Brown et al 
(2000), in which inhibition of entry of physiologic adenosine 
analogs (adenosine, AMP, ADP, and ATP) into human keratino-
cytes prevented the antiproliferative activity of these agents.
Another similarity between our results and the data obtained
with adenosine analogs is the reversibility of the effects (Fig 2 and Cook et al, 1995). Nevertheless, there are distinct differences as well. For instance, Brown et al (2000) found that adenosine analogs arrested cells in S phase, whereas we observe 8-Cl-adenosine-induced arrest primarily in G0/G1 (Tables I, II). These disparities may or may not be due to differences in methodology (24 h vs 48 h incubations with agents and/or varying degrees of subconfluence) or species (mouse versus human). Cook and colleagues (Cook et al, 1995; Brown et al, 2000) also did not determine the effects of the adenosine analogs on markers of keratinocyte differentiation. Thus, it is not clear whether 8-Cl-adenosine inhibits growth via a novel mechanism or through one similar to that utilized by physiologic adenosine analogs.

Phase II trials of 8-Cl-cAMP for treatment of human cancers are in progress (McDaid and Johnston, 1999). The mechanism of 8-Cl-cAMP inhibition of cancer cell proliferation is unknown but is likely to involve conversion of 8-Cl-cAMP to 8-Cl-adenosine (Van Lookeren Campagne et al, 1991; Han et al, 1993; Scala et al, 1995; Halgren et al, 2001; Yin et al, 2001). Similarly, 8-Cl-cAMP induces apoptotic cell death in some cancer cell lines through its 8-Cl-adenosine metabolite (Halgren et al, 2001; Yin et al, 2001). On the other hand, we have found that 8-Cl-cAMP is less potent than 8-Cl-adenosine at inhibiting normal keratinocyte proliferation (data not shown). In addition, our results suggest that 8-Cl-adenosine instead causes a reversible G0/G1 growth arrest. Therefore, whereas 8-Cl-adenosine does not induce apoptosis in normal keratinocytes but instead causes a reversible G0/G1 growth arrest, therefore, whereas 8-Cl-adenosine may temporarily inhibit keratinocyte proliferation, the reversibility of this effect should preclude any long-term negative consequences. Indeed, in athymic mice in vivo administration of 8-Cl-adenosine inhibits tumor formation by a colorectal cancer cell line without significant toxicity (Carlson et al, 2000). Thus, by sparing the stem cells and/or transit-amplifying cells of the epidermis while triggering apoptosis in cancer cells, both 8-Cl-cAMP and its metabolite 8-Cl-adenosine should exhibit little skin toxicity and hopefully prove to be a useful therapy for treatment of human cancers.

Takeda et al argue (Takeda et al, 1994) that 8-Cl-adenosine can inhibit growth of a nontransformed/nonimmortalized cell, primary mouse keratinocytes, and (ii) this growth inhibition is dissociated from the induction of the keratinocyte differentiation program. Thus, 8-Cl-adenosine represents a small nonpeptide molecule that, like TGF-β, is capable of arresting keratinocyte growth without eliciting differentiation. Finally, it should be noted that careful consideration must be applied when investigating the apparent cell specificity and mechanisms by which the antiproliferative effects of 8-Cl-adenosine are exerted.


Gliner RD, Bellag WB: Inhibition of [3H]thymidine transport is a nonspecific effect of PDDP in primary cultures of mouse epidermal keratinocytes. J Pharmacol Exp Ther 294:1219–1224, 2000


Han Z, Chatterjee D, Wyche JH: Proliferation of nontransformed cells is inhibited by 8-Cl-adenosine metabolite of but not by parental 8-Cl-cyclic AMP. J Pharmacol Exp Ther 265:790–794, 1993


REFERENCES


Bikle DD, Pillaí S: Vitamin D, calcium and epidermal differentiation. Endocrine Rev 14:5–19, 1993

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