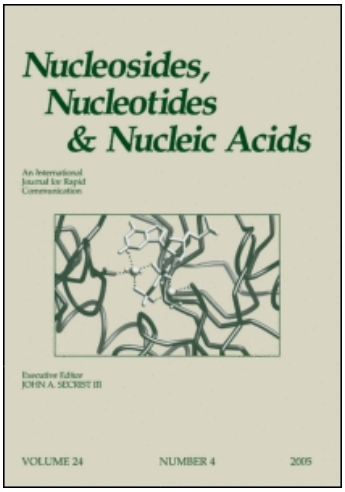

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Inhibition of Cell Growth and Proliferation in Human Glioma Cells and Normal Human Astrocytes Induced by 8-Cl-cAMP and Tiazofurin

M. Pesic[a]; K. Drabek[b]; C. Esler[b]; S. Ruzdijic[b]; V. Pejanovic[b]; Z. Pietrzkowski[b]
[a] Department of Neurobiology and Immunology, Laboratory of Molecular Neurobiology, Institute for Biological Research, Belgrade, Yugoslavia [b] ICN Pharmaceuticals, R & D, Costa Mesa, California, USA

To cite this Article Pesic, M. , Drabek, K. , Esler, C. , Ruzdijic, S. , Pejanovic, V. and Pietrzkowski, Z.(2000) 'Inhibition of Cell Growth and Proliferation in Human Glioma Cells and Normal Human Astrocytes Induced by 8-Cl-cAMP and Tiazofurin', Nucleosides, Nucleotides and Nucleic Acids, 19: 5, 963 — 975
To link to this Article: DOI: 10.1080/15257770008033036
URL: http://dx.doi.org/10.1080/15257770008033036

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 19(5&6), 963–975 (2000)

INHIBITION OF CELL GROWTH AND PROLIFERATION IN HUMAN GLIOMA CELLS AND NORMAL HUMAN ASTROCYTES INDUCED BY 8-Cl-cAMP AND TIAZOFURIN

♦*M. Pesic, ♦#K. Drabek, #C. Esler, S. Ruzdijic, #V. Pejanovic, #Z. Pietrzkowski

Institute for Biological Research, Department of Neurobiology and Immunology, Laboratory of Molecular Neurobiology, 29. Novembra 142, 11060 Belgrade, Yugoslavia; # ICN Pharmaceuticals, R&D, 3300 Hyland Avenue, Costa Mesa, California 92626, USA; ♦ These authors contributed equally to this work.

ABSTRACT: 8-Cl-cAMP and tiazofurin (TR) are anti-tumor agents that besides their antiproliferative effect, also induce differentiation of tumor cells. Although, these agents exert a profound effect on the same events of tumor cell life, it is thought that 8-Cl-cAMP and TR act by modulating the signal transduction pathway through distinct mechanisms. We have compared their effect on two human glioma cell lines (U87 MG and U251 MG) and examined if there is selectivity in their action toward normal human astrocytes.

INTRODUCTION

One of the anticancer strategies is enzyme directed chemotherapy. It is based on a fact that among other alterations animal and human cancer cells are characterized by a strongly conserved, ordered pattern of imbalance in carbohydrate, purine, pyrimidine, amino acid, polyamine and cyclic AMP metabolism.[1] Key enzymes in these metabolic pathways represent potential targets for anticancer drugs to attack.

Cancer cells tend to have high rates of nucleic acid biosynthesis and higher levels of the enzymes involved in these pathways. The first enzyme in the guanine biosynthesis pathway is inosine monophosphate dehydrogenase (IMPDH). This enzyme should be a sensitive target for the design of rational chemotherapy. Two IMPDH isoforms are found in mammalian cells.[2] In normal tissues, IMPDH type I is expressed constitutively and has the lowest activity among all enzymes of purine biosynthesis or degradation; IMPDH

type II is elevated in tumors in a transformation- and progression- linked fashion; IMPDH is the rate-limiting enzyme of *de novo* guanosine triphosphate (GTP) biosynthesis and it also governs the production of guanosine diphposphate (GDP) from which deoxy-GDP is produced by ribonucleotide reductase.[3]

A series of inhibitors designed to block IMPDH enzymatic activity was synthesized.[4] One of the compounds synthesized, tiazofurin (2-β-D-ribofuranosyl-thiazole-4-carboxamide), proved to be a potent inhibitor of IMPDH and a novel anticancer drug. The mechanism of action of tiazofurin (TR) is due to its metabolic conversion to active metabolite 4-thiazole-carboxamide adenine dinucleotide (TAD), an analog of nicotinamide adenine dinucleotide (NAD).[5] TAD tightly bounds at the NADH site of IMPDH enzyme inhibiting its activity. The inhibition leads to decreased GTP concentration, causing inhibition of DNA and RNA biosynthesis and cell proliferation. There is a direct relationship between cellular uptake of TR, its conversion to TAD in target cells and its effectiveness.[6]

Adenosine 3',5'-monophosphate (cAMP) acts in mammalian cells by binding to either of two distinct isoforms of protein kinase A (PKA), termed PKA I and PKA II. PKA I and PKA II have identical catalytic subunits but differ in the regulatory subunits (defined as R I in PKA I and R II in PKA II). Differential expression of PKA I and PKA II has been correlated with cell differentiation and neoplastic transformation. In fact, preferential expression of PKA II is found in normal nonproliferating tissues and in growth-arrested cells, whereas enhanced levels of PKA I are detected in tumor cells and in normal cells following exposure to mitogenic stimuli.[7]

The potency of 8-Cl-cAMP (one of C-8 adenosine 3',5'-monophosphate analogs)-induced growth inhibition correlates with its selective modulation of protein kinase A isozymes (PKA I versus PKA II). In the activation of PKA, 8-Cl-cAMP showed 3.3-fold greater potency for PKA I than for PKA II.[8]

Several hundred analogs of cyclic nucleotides have been synthesized and tested, but no one has reported a cyclic nucleotide that demonstrates growth inhibition against the broad spectrum of human cancer cells as well as was shown with 8-Cl-cAMP. The growth arrest and differentiation in a variety of human cancer cell lines are accompanied by an increased R II/ R I (PKA regulatory subunits) ratio and by inhibition of expression of different oncogenes and growth factors.[8]

R IIβ cAMP receptor protein possesses the specific amino acid sequence of nuclear location and thus translocates rapidly into the cell nucleus upon stimulation with 8-Cl-cAMP where enhances formation of the CRE-nuclear protein complex. Therefore, R IIβ may be the modulator of gene transcription involved in the regulation of cell proliferation and differentiation.[9] However, some authors have suggested that the inhibitory effect of 8-Cl-cAMP might not be a true 8-Cl-cAMP effect, but rather the effect of 8-Cl-adenosine, which is a major metabolite of 8-Cl-cAMP.[10]

Considering the resistance of human gliomas to chemotherapeutic treatment, there is a continuos need for developing new strategies that would provide better rate of patients survival and life quality.

As both 8-Cl-cAMP and TR are thought to act by modulating the signal transduction pathway through distinct mechanisms, in this study, we have compared their effect on two human glioma cell lines and their selectivity toward normal human astrocytes (NHA).

MATERIALS AND METHODS

Cell lines

Two different glioma cell lines (U87 MG and U251 MG) were studied. Glioma cell line U87MG was obtained from the ATCC (Rockville, MD). U251 MG glioma line was provided by Dr. Martin Haas, University of California San Diego, Department for Biology, La Jolla, CA. Astrocyte culture was established at Clonetics Corporation's cell culture facility from normal human tissue.

Cell culture

U87 MG was cultured in Minimum Essential Medium Eagle Mod. (MEM) supplemented with heat inactivated fetal bovine serum-FBS (10% v/v), L-glutamine (2 mM), penicillin (50 UI/ml) and streptomycin (50 μg/ml) (all from ICN Pharmaceuticals, Costa Mesa, CA). U251 MG was cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with heat inactivated calf serum-CS (10% v/v), L-glutamine (2 mM), penicillin (50 UI/ml) and streptomycin (50 μg/ml) (all from ICN Pharmaceuticals, Costa Mesa, CA). Both glioma cell lines were also cultured, as well as Normal Human Astrocytes (NHA), in Astrocyte Basal Medium (ABM) supplemented with FBS (5% v/v), progesterone (25 μg/ml), transferrin (50 mg/ml), insulin (10 mg/ml), hEGF (human

recombinant Epidermal Growth Factor, 10 μg/ml), gentamicin (50 mg/ml) and amphotericin-B (50 μg/ml) (all from Clonetics Corporation, Walkersville, MD). Cells were subcultured at 72 hr intervals using 0.25% trypsin/EDTA (Clonetics), and seeded into fresh medium at 1:15. Cultures were incubated at 37^{0}C in a humidified atmosphere of 5% CO_2 and 95% air.

8-Cl-cAMP and tiazofurin (TR)

8-Cl-cAMP (8-Chloroadenosine-3',5'-monophosphate) and tiazofurin (2-β-D-ribofuranosyl-thiazole-4-carboxamide) were synthesized in R&D, ICN Yugoslavia.

DNA synthesis assay

Cells were seeded into 96-well plates at 4 x 10^4 cells per well and treated with different concentrations of 8-Cl-cAMP and TR. The plates were incubated for 20, 44 and 68 h, after which [^{3}H]-thymidine (ICN Pharmaceuticals) was added to each well to a final concentration of 1 μCi/ml. Cells were incubated for 4 h to allow incorporation of [^{3}H]-thymidine into cellular DNA. They were then lysed with 0.25% trypsin/EDTA (Clonetics) for 15 min. Cell lysate was harvested (Harvester 96® Tomtec) and counted using 1205 Betaplate Liquid Scintillation Counter (Wallac). Incorporation of [^{3}H]-thymidine into cell DNA was expressed as CCPM (corrected CPM), average ± STDEV of at least triplicate determinations.

Growth inhibition assays

For growth-inhibition studies, cells were seeded at 2 x 10^4 cells per well in 96-well plates and treated with different concentrations of 8-Cl-cAMP and TR for 72 h. Viable cell number was determined using two different assays: 1) MTS cell proliferation/cytotoxicity assay (Promega, Forward, WI), in which the capacity of cells to convert a tetrazolium salt (3-4,5-dimethylthiazol-2-5-3-carboxymethoxyphenyl-2-4-sulfophenyl-2H-tetrazolium) to formazan was measured. The absorbance at 490 nm represents the number of metabolically active cells in culture.[11] 2) NRU (Neutral Red Uptake) assay (Clonetics), in which the capacity of cells to concentrate Neutral Red dye (3-amino-7-dimethylamino-2-methylphenazine hydrochloride) in their lysosomes was measured. The absorbance at 540 nm represents the number of cells with preserved

membrane integrity.[12] The results from both assays were obtained using ELISA Reader (Titertec Multiscan Bichromatic, ICN Pharmaceuticals). All values were assessed at least in triplicate.

Determination of cell viability

Trypan blau dye exclusion test was performed for determination of 8-Cl-cAMP and TR cytotoxicity. Treated cells, as well as untreated, were collected in 200 µl medium volume and mixed with 300 µl PBS (phosphate buffer saline, ICN Pharmaceuticals) and 500 µl of 0.4% trypan blau (Sigma) dye solution. The cells were counted in Burker's chemocytometer under the invert microscope (Zeiss). The death cells were colored blue, while viable cells staid uncolored. The counting was performed for each treatment at least in triplicate.

Method of data analysis

All results were expressed as the averages ± standard deviations (STDEVs). The Student *t*-test (two-tailed) was utilized to compare and evaluate the statistical significance of the data. A *p*-value of less than 0.05 was considered to indicate a significant difference. IC_{50} values were obtained with FORECAST function in Microsoft Excel.

RESULTS

Effect on [^{3}H] thymidine uptake

8-Cl-cAMP and TR have decreased [^{3}H] thymidine incorporation in both glioma cell lines in a dose-related fashion. Untreated U87 MG and U251 MG cells have shown 231 % and 96 % increased (respectively) [^{3}H] thymidine incorporation after 72 h when they were cultured in Astrocyte Growth Medium-AGM (data not shown). Despite rapid growth of those cell lines in AGM medium, the effect of 8-Cl-cAMP and TR remained in dose-related manner (Fig. 1).

Median inhibitory concentration (IC_{50}) values for 8-Cl-cAMP in regular medium-RM (MEM for U87 MG and DMEM for U251 MG) and AGM media were similar on both cell lines which indicate that the effect of this drug was not diminished in AGM medium (Table 1). TR has shown even better effect on U87 MG cells cultured in AGM medium (Table 1).

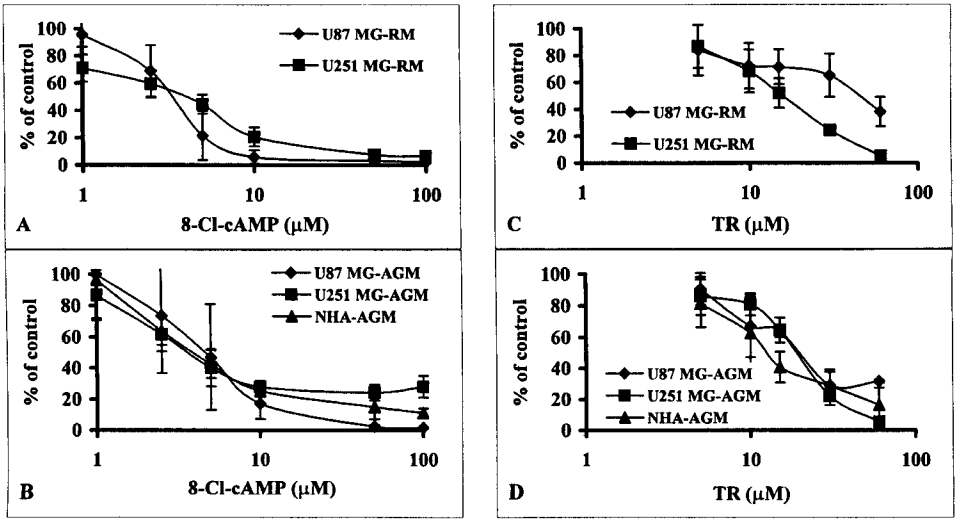

FIG. 1. The effect of different concentrations of agents on the rate of [^{3}H] thymidine incorporation in tested cells, after 72 h of incubation. A: Effect of 8-Cl-cAMP on U87 MG and U251 MG cells cultured in RM medium. B: Effect of 8-Cl-cAMP on U87 MG, U251 MG cells and NHA cultured in AGM medium. C: Effect of TR on U87 MG and U251 MG cells cultured in RM medium. D: Effect of TR on U87 MG, U251 MG cells and NHA cultured in AGM medium. The percent of control was obtained as (C/C0), where (C0) represents CCPM of untreated cells and (C) represents CCPM of cells treated with different concentrations.

TABLE 1. Value of IC_{50} for [^{3}H] thymidine incorporation in glioma cell lines and NHA.*
*IC_{50} (μM) = median inhibitory concentration

	U87 MG		U251 MG		NHA
	RM	AGM	RM	AGM	AGM
8-Cl-cAMP	3.5 ± 0.6	4.7 ± 1.6	4.1 ± 0.3	3.9 ± 0.5	4.1 ± 0.4
TZF	46.8 ± 5.1	21.1 ± 0.8	16.0 ± 1.8	20.2 ± 1.6	12.9 ± 3.0

There was no significant statistical difference in the effect of tested drugs among human glioma cell lines and normal human astrocytes. The glioma cell lines and normal human astrocytes-NHA have shown IC_{50} values for 8-Cl-cAMP between 3.5 and 4.7 μM. IC_{50} values for TR were higher (Table 1).

When compared with untreated cells, the effect of 8-Cl-cAMP on [^{3}H] thymidine incorporation was statistically significant at 1 μM ($p < 0.05$) for both cell lines, as well as for NHA. TR has shown statistically significant effect at 5 μM ($p < 0.05$).

The effect on [^{3}H] thymidine incorporation (Fig. 1: A and B) has shown that 8-Cl-cAMP has similar effect on tested cell lines, by reaching the plateau with 10 μM. TR has shown different dose-related profiles in comparison with 8-Cl-cAMP (Fig. 1: C and D), without reaching the plateau even with highest concentration (60 μM). Also, U87 MG cells were remarkable resistant on TR action in RM medium in comparison with U251 MG cells (Fig. 1:C).

Treatment with IC_{50} concentrations (Table 1) at each time point (4, 24, 48 and 72 h) has shown that the effect of 8-Cl-cAMP is time-dependent on both glioma cell lines. TR had the best effect after 24 h, while after 72 h of treatment the cell proliferation was significantly recovered (Fig. 2).

Effect on cell growth

The effect of 8-Cl-cAMP and TR on cell growth accessed with NRU assay was dose-dependent on both glioma cell lines, as well as on NHA. There was no significant difference in the effect on cell growth with tested drugs among glioma cell lines and NHA (Fig. 3).

IC_{50} values for NHA were over the highest tested concentrations for both drugs. 8-Cl-cAMP has shown the strongest effect on U87 MG with IC_{50} value 49.5 μM, whereas U251 MG was less sensitive with IC_{50} over the highest tested concentration. IC_{50} values for both glioma cell lines were not reached with highest concentration of TR (60 μM). U87 MG cells treated with TR have shown similar resistant profile for growth inhibition as well as for inhibition of [^{3}H] thymidine incorporation (Fig. 1: C and Fig. 3: B).

Lower concentrations of 8-Cl-cAMP have shown strong potential for growth inhibition (Fig. 3: A), but higher concentrations did not elevate that effect as was expected knowing the profiles for [^{3}H] thymidine incorporation (Fig. 1: A and B).

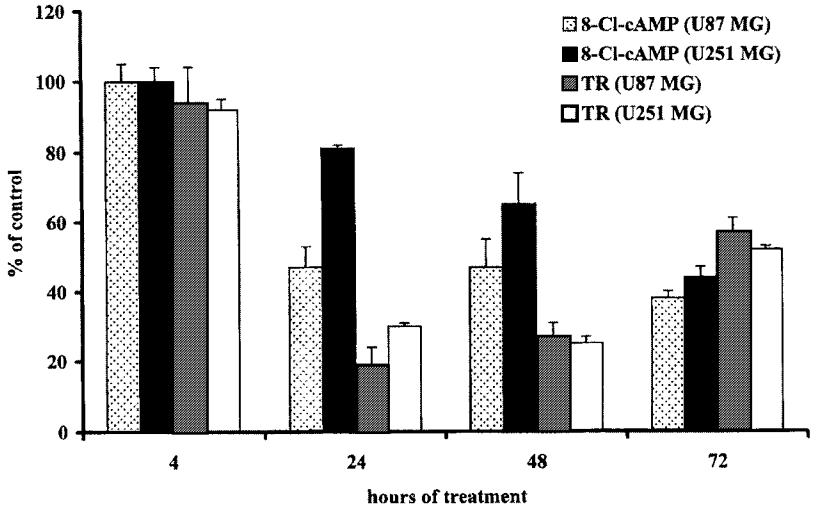

FIG. 2. The effect of IC_{50} concentrations of 8-Cl-cAMP and TR on both glioma cell lines at different time points, accessed by [^{3}H] thymidine uptake.

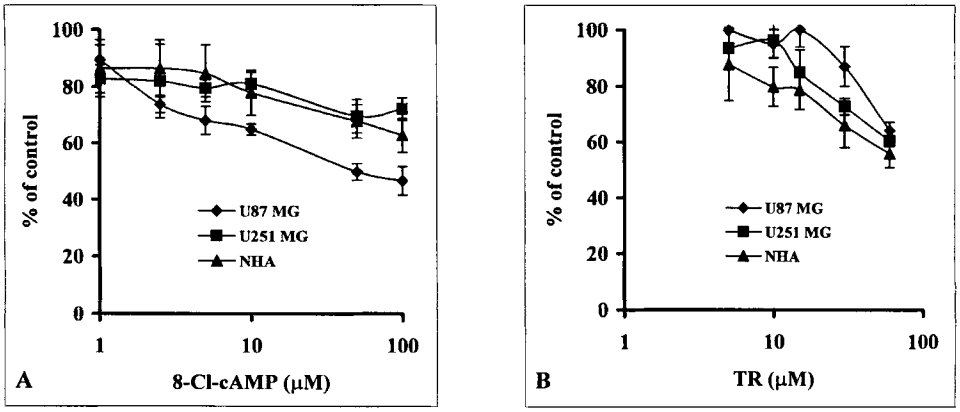

FIG. 3. A: The effect of 8-Cl-cAMP on cell growth measured by the capacity of cells to concentrate Neutral Red dye in their lysosomes. B: The effect of TR on cell growth measured by the capacity of cells to concentrate Neutral Red dye in their lysosomes. The percent of control was obtained as (A/A0) , where (A0) represents absorbance at 540 nm of untreated cells and (A) represents absorbance at 540 nm of cells treated with different concentrations.

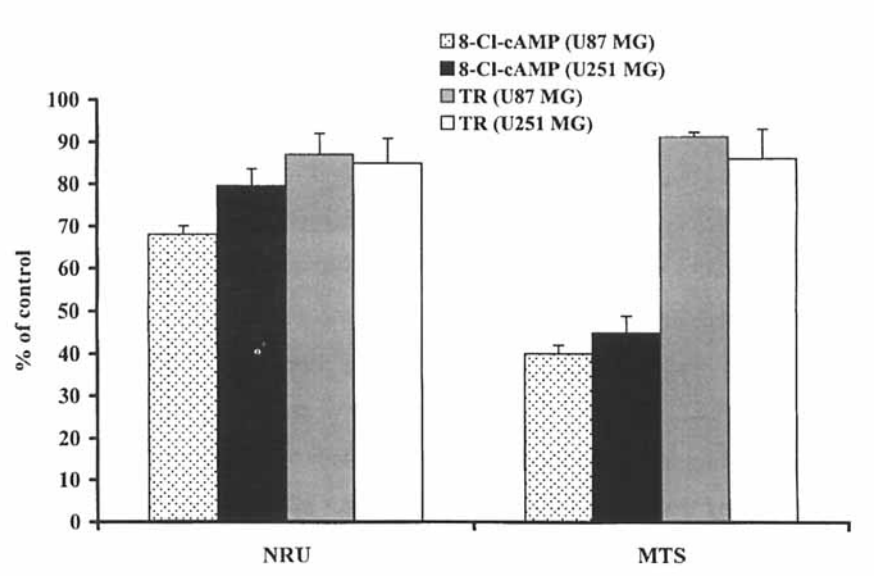

FIG. 4. The effect of IC_{50} concentrations of 8-Cl-cAMP and TR on the inhibition of glioma cell growth measured by two different assays (NRU and MTS).

The effect of 8-Cl-cAMP on glioma cell growth was stronger detected with MTS than with NRU assay. In case of TR, there was no difference between results obtained with those two assays. Also, 8-Cl-cAMP has shown stronger inhibitory effect on both glioma cell lines in comparison with TR (Fig. 4).

Effect on cell viability

Treatments with 8-Cl-cAMP and TR did not decrease cell viability in comparison with untreated cells. The cell viability assessed with trypan blau dye exclusion assay was about 90% even with the highest concentrations of 8-Cl-cAMP and TR.

DISCUSSION

More and more data are available showing that TR, besides its antiproliferative effect, also exerts a profound effect on several other events of cell life, such as apoptosis[13, 14] and cell differentiation.[15] TR down-regulates *ras* and *myc* oncogenes, as well as G-protein mediated transmembrane signaling pathways. Biochemical studies have indicated that TR

inhibits the glycosylation of a wide range of glycoproteins, with the most profound effect on proteoglycans.[3] It down-regulates signal transduction activity due to a reduction of the activities of 1-phosphatidylinositol 4-kinase (PI) and 1-phosphatidylinositol 4-phosphate 5-kinase (PIP) leading to a decrease in the concentration of the second messenger, inositol 1, 4, 5-triphosphate (IP3).[5]

The growth inhibition induced by 8-Cl-cAMP brought about various effects among the cell lines tested, including biochemical (oncogene and transforming-growth-factor α suppression) and morphological changes, differentiation, and reverse transformation.[15] Different morphological and biochemical criteria have demonstrated that 8-Cl-cAMP and its major metabolite, 8-Cl-adenosine, induce cell death by apoptosis in malignant cells.[16] Besides, it appears that 8-Cl-cAMP produces growth inhibition while allowing the cells to progress through their normal cell cycle, albeit at a slower rate, and this may lead to eventual restoration of a balance between cell proliferation and differentiation in cancer cells.[17] 8-Cl-cAMP also reverses the MDR (multidrug resistance) phenotype by acting as a selective antagonist of major PKA isoform present in MDR cells, PKA I.[18]

Our results have shown that both agents inhibit the cell proliferation of the examined glioma cell lines with IC_{50} values in the micromolar range, with 8-Cl-cAMP being markedly more active (Table 1). There was no selectivity in 8-Cl-cAMP and TR action toward primary culture of human astrocytes (NHA) (Table 1). Similar effect of tested agents on tumor and normal cells *in vitro* (Fig. 1) could be explained due to specific conditioning of NHA in AGM medium. The proliferation of normal cells was induced with growth factors and hormones, so 8-Cl-cAMP and TR could act on their rate of proliferation through the similar mechanisms as in tumor cells. Because of that specific conditioning, NHA model system didn't reflect *in vivo* situation.

The strong biological potential of 8-Cl-cAMP and TR is evident from the fact that their inhibitory effect on both glioma cell lines was not diminished in AGM despite rapid cell growth in this medium (Table 1). For the first set of experiments we have chosen only one glioma cell line (U87 MG), but we have found out that this line cultured in RM medium was resistant on TR action. In next step, we have decided to examine the behavior of U87 MG cells in AGM. Also, we wanted to compare the effect of 8-Cl-cAMP and TR on U87 MG with the effect on U251 MG cells that were more sensitive on TR action. The obtained results indicate that U87 MG cells became more sensitive on TR

action when they were cultured in AGM. IC_{50} value for U87 MG in RM was 46.8 μM, while IC_{50} value obtained in AGM was 21.1 μM. This phenomenon could be explained due to growth-promoting features of AGM. In case of U87 MG cells, it could change their inherent properties by making them more sensitive on TR action. Also, U87 MG cell line had longer cell duplication time (59 ± 3.4 h) in comparison with U251 MG (41.4 ± 3.4 h).

The effect on cell growth was less intensive than the effect on [^{3}H] thymidine incorporation which could be explained with regiment of treatment (Fig. 1 and Fig. 3). The cells were treated only once and the effect was measured after 72 h. Other authors have shown stronger effect on cell growth with these agents, but with prolonged and repeated treatments.[19-22]

The effect of 8-Cl-cAMP on cell proliferation was time-dependent with the best effect after 72 h of treatment (Fig. 2). These results are in accordance with new data which claim that the growth inhibition by 8-Cl-cAMP is accompanied with almost complete elimination of PKA I isozyme, without effecting PKA II. Also, 8-Cl-cAMP induces an arrest in the G0/G1-phase of the cell cycle.[23]

TR has shown the strongest antiproliferative effect on both glioma cell lines after 24 h (Fig. 2). TR effect through time was diminished and cell proliferation was partially recovered after 72 h (Fig. 2). This could be explained with rapid TR action against *de novo* synthesis of guanine nucleotides in U87 MG and U251 MG cells and possible engagement of salvage pathways [6] of synthesis after prolonged treatment in these glioma cells.

Stronger effect of 8-Cl-cAMP obtained with MTS assay in comparison with NRU (Fig. 4) has shown that 8-Cl-cAMP impacts metabolic processes in cells rather than disturbing their plasma membrane integrity.

In this study about 90 % of cells of both glioma cell lines, as well as NHA, excluded trypan blue, even after 72 h of treatment with highest concentrations, indicating that 8-Cl-cAMP and TR were not cytotoxic under these experimental conditions.

Since it was shown that IMPDH activity in glioblastoma is 4-fold higher than in normal brain tissue,[24] modulation of the guanylate pathway in glioblastoma by inhibition of IMPDH with tumor specific agents such as TR could be a rational therapeutic approach.

Clinical studies have shown that after treatment with nontoxic doses of 8-Cl-cAMP, the concentration level in patients blood plasma riches 0.05-5 μM, which corresponds with IC_{50} concentrations obtained on human tumor cells *in vitro*.[25]

Considering those data[24, 25] and the antiproliferative effect on human glioma cells shown in this study, 8-Cl-cAMP and TR might provide an interesting approach towards a more effective treatment of human gliomas.

Acknowledgements. *Strategic&Technological Project (ID-58) from the Ministry of Science and Technology, Republic of Serbia supported this work.*

REFERENCES

1. Weber, G. Cancer Res. **1983**, *43*, 3466-3492.

2. Franchetti, P.; Grifantini, M. Curr. Med. Chem. **1999**, *6*, 599-614.

3. Boritzki, T. J.; Berry, D. A.; Besserer, J. A.; Cook, P. D.; Fry, D. W.; Leopold, W. R.; Jackson, R. C. Biochem. Pharmacol. **1985**, *34*, 1109-1114.

4. Robins, R. K. Nucleosides and Nucleotides. **1982**, *1*, 35-44.

5. Weber, G.; Shen, F.; Prajda, N.; Yeh, Y. A.; Yang, H.; Herenyiova, M.; Look, K. Y. Anticancer Res. **1996**, *16*, 3271-3282.

6. Jackson, R. C.; Harkrader, R. J. In Nucleosides and Cancer Treatment, ed. by M. H. N. Tattersall and R. M. Fox. Sydney: Academic Press. **1981**, 18-31.

7. Cho-Chung, Y. S. Cancer Res. **1990**, *50*, 7093-7100.

8. Ally, S.; Tortora, G.; Clair, T.; Grieco, D.; Merlo, G.; Katsaros, D.; Ogried, D.; Doskeland, S. O.; Jahnsen, T.; Cho-Chung, Y. S. Proc. Natl. Acad. Sci. USA. **1988**, *85*, 6319-6322.

9. Mednieks, M. I.; Yokozaki, H.; Merlo, G. R. FEBS Lett. **1989**, *254*, 83-88.

10. Van Lookeren Campagne, M. M.; Diaz, F. V.; Jastorff, B.; Kessin, R. H. Cancer Res. **1991**, *51*, 1600-1605.

11. Goodwin, C. J.; Holt, S. J.; Downes, S.; Marshall, N. J. J. Immunol. Methods **1995**, *179 (1)*, 95-103.

12. Wallace, K. A.; Harbell, J. W.; Janus, J.; Curren, R. D. In Vitro Cell. Develop. Biology **1993**, *29 (3)*, 98.

13. Vitale, M.; Zamai, L.; Falcieri, E.; Zauli, G.; Gobbi, P.; Santi, S.; Cinti, C.; Weber, G. Cytometry. **1997**, *30*, 61-66.

14. Piperski, V.; Vracar, M..; Jokanovic, M.; Stukalov, P.; Rakic, L. Apoptosis. **1998**, *3*, 345-352.

15. Ruzdijic, S.; Kanazir, S.; Milosevic, J.; Papovic, M.; Drabek, K.; Medic-Mijacevic, LJ.; Pejanovic, V.; Rakic, LJ. Arch. Pharm. **1998**, *5*, 419-435.

16. Boe, R.; Gjersten, B. T.; Doskeland, S. O.; Vintermyr, O. K. Brit. J. Cancer. **1995**, *72(5)*, 1151-1159.

17. Cho-Chung, Y. S.; Clair, T. Pharmac.&Therap. **1993**, *60(2)*, 265-288.

18. Scala, S.; Budillon, A.; Zhan, Z.; Cho-Chung, Y. S.; Jefferson, J.; Tsokos, M. The J. Clin. Invest. **1995**, *96*, 1026-1034.

19. Jayaram, H. N.; Murayama, K.; Pillwein, K.; Zhen, W.; Weber, G. Cancer Chemother. Pharmacol. **1992**, *31*, 93-96.

20. Olah, E.; Csokay, B.; Prajda, N.; Kote-Jarai, Z.; Yeh, Y. A.; Weber, G. Anticancer Res. **1996**, *16*, 2469-2478.

21. Ciardiello, F.; Tortora, G.; Kim, N.; Clair, T.; Ally, S.; Salomon, D. S.; Cho-Chung, Y. S. The J. Bio. Chem. **1990**, *265(2)*, 1016-1020.

22. Cho-Chung, Y. S.; Clair, T.; Tagliaferri, P.; Ally, S.; Katsaros, D.; Tortora, G.; Neckers, L.; Avery, T. L.; Crabtree, G.W. Robins, R. K. Cancer Invest. **1989**, *7(2)*, 161-177.

23. Noguchi, K.; Murata, T.; Cho-Chung, Y. S. Eur. J. Cancer. **1998**, *34(8)*, 1260-1267.

24. Pillwein, K.; Chiba, P.; Knoflach, A.; Czermak, B.; Schuchter, K.; Gersdorf, E.; Ausserer, B.; Murr, C.; Goebl, R.; Stockhammer, G.; Maier, H.; Kostron, H. Cancer Res. **1990**, *50*, 1576-1579.

25. Tortora, G.; Ciardiello, F.; Pepe, S.; Tagliafferri, P.; Ruggiero, A.; Bianco, C.; Guarrasi, R.; Miki, K.; Bianco, A. R. Clin. Cancer Res. **1995**, *1*, 377-384.

Received 10/12/99
Accepted 2/29/00