# 8-Cl-cAMP, "The Old Dog with New Tricks": A Review

Vladan Bajić[a,*], Lada Živković[b], Andrea Čabarkapa[a] and Biljana Spremo Potparević[a]

[a]*Laboratory for Radiobiology and Molecular Genetics, Institute for Nuclear Research "Vinca", University of Belgrade, Serbia*

[b]*Department of Physiology, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia*

**Abstract:** Current chemotherapeutic drugs act for the most part by killing cancer cells directly. Treatment with these drugs often can be harmful to normal cells and may cause incomplete elimination of the target cells, resulting in the recurrence of the disease.

To coop with current treatments the path of biomodulation rather than cytotoxicity, has been seen in the role of cAMP in normal *versus* malignant cells. It has been found that an increase of cAMP levels in normal cells stimulates proliferation, and that in the same time cancerous cells are inhibited to proliferate. This inverted reaction has given the momentum for synthesis of various cAMP analogues and investigation of there antitumor activity. A number of analogues, such as 8-PIP-cAMP, 8-Br-CAMP or 8-HA–cAMP showed efficacy only in millimolar concentrations. Only one of them, 8-Cl-cAMP as specific analogue has achieved inhibition of proliferation and stimulation of apoptosis of malignant cells in low or micromole concentrations.

Still, 25 years later the mechanism of action of 8-clcAMP has not been fully elucidated or defined. This review is to challenge these mechanisms of action and to set a view of the nature of 8-Cl-cAMP action.

**Keywords:** 8-Cl-cAMP, PKA cAMP-dependent protein kinase, biomodulation.

## INTRODUCTION

Cancer cells are characterized by an arrest at an incomplete stage of maturation while retaining their capacity to proliferate. Several agents act by changing the biological properties of cancer cells so they lose their ability to divide continuously. Once this occurs, the cells are programmed to die. This new approach is based on the demonstration that cancer cells can differentiate to maturity and therefore reach a stage of development in which division ceases. These findings counter other views that cancer cells are locked irrevocably in an immature state that allows them to proliferate uncontrollably [1, 2].

Although cancer cells have the characteristics of immaturity, that condition is not necessarily permanent. How does the process of biomodulation occur is still a matter of debate. Here in this mini review we will present a promising agent, 8-ClcAMP, a site selective cAMP analogue that has as a class of biomodulators endured a long period of pre and clinical research [1]. Its biomodulator properties that primary acts by modulating cells to go from an immature state to a more differentiated state thus taking cells into a more controlled state, regaining the property of cell death-*apoptosis*, in our view, has been and is a promising strategy for cancer treatment. From the time of the discovery of cAMP by Sutherland in 1957 as a mediator of hormonal signals in all cells and tissues an idea to modulate intracellular signals for restoring normal controlling mechanisms in malignant cells has been born. This lead to the synthesis of a number of cAMP analogues, but until 8-Cl-cAMP introduction in main stream research 25 years ago [1] no real shift has been made into the process of bio-modulating malignant cells.

The biomodular activity acts through the regulation of cAMP dependent protein kinase A (PKA). 8-Cl-cAMP modifies the ratio of the PKA regulatory (R) subunits (type I vs. type II) by decreasing the levels of type I R subunits and by dissociation cAMP that binds differentially to site 1 versus site 2 [1]. Thus, cAMP analogues modified at the C-2 or C-8 position on the adenine ring (C-2 and C-8 analogs, respectively) are generally selective for Site 1, whereas analogs modified at the C-6 position (C-6 analogs) are generally selective for Site 2 (for classification of analogues see review [1, 3]).

Recently, Lucchi *et al*., [3] and Dictore *et al*., [4] using 8 Cl-cAMP and site-specific cAMP analogs 8-piperidinoadenosine-3',5'-cyclic monophosphate (8-PIP-cAMP) and 8-hexylaminoadenosine-3',5'-cyclic monophosphate (8-HA-cAMP) (8-PIP-cAMP and 8-HA-cAMP) in BRAF-positive carcinoma cell lines and MTC cell lines, respectively showed 8-Cl-cAMP and the PKA I-selective cAMP analogs a potent anti-proliferative effect in both cell lines. 8-Cl-cAMP appears to be

*Address correspondence to these authors at The Institute of Nuclear Sciences "Vinča", Laboratory for Radiobiology and Molecular Genetics, P.O. Box 522. 11001 Belgrade, Serbia; Tel: +381113408147; Fax: +381116447485;
E-mail: vladanbajic@yahoo.com

ISSN: 1929-2260 / E-ISSN: 1929-2279/15 

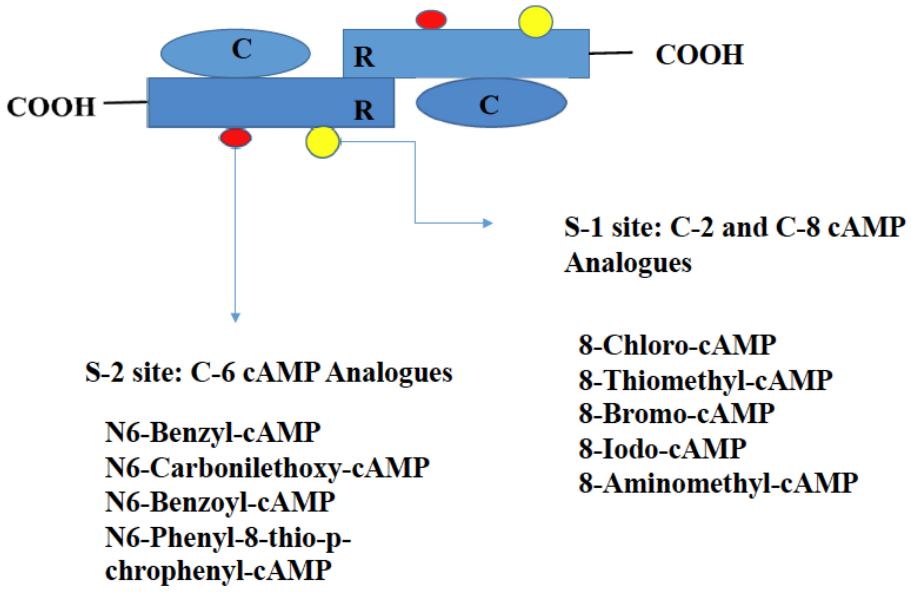


**Figure 1:** A representation of binding sites on cAMP –dependent protein kinase with representative cAMP binding analogues.

Represents the process of cAMP selectivity that is based on modifications at the C-2 and C-8 position of the adenine ring. The C-8 analogues are generally selective for site 1 and vice versa. The figure gives a representation of both isoforms of the PKA protein. 8-Cl-cAMP has been attributed to its ability to discriminate between the two isoforms of PKA, type I PKA versus type II PKA. Because of its low affinity to activate type II PKA, the ratio of type I versus type II is lowered thus resulting in the restoration of "normal" PKA protein levels in tumour cells thus inducing a reversion to a ‘‘normal’’ phenotype (*Biomodulation)*.

effective in a broader range of cell types and induces apoptosis. On the other hand, the mechanism(s) of action of 8-Cl-cAMP and the PKA I-selective cAMP analogs are different, suggesting that they may have dissimilar pharmacological and toxicological profiles, as well as unique antitumor properties.

This pro-apoptotic effect of 8-Cl-cAMP is accompanied by an increase in the p38MAPK pathway and its effects are triggered by its conversion to its metabolite 8-Cl-adenosine [4].

## Biomodulation through 8-ClcAMP *versus* 8-Cl-Adenosine

One of the major problems is the overall debated whom or what can be identified as the major players for its antitumor action. In regard that 8-ClcAMP is a highly specific analogue of cAMP it is logically that this agent would be recognized as substrate of the enzymes that are part of the cAMP metabolism. So, one group of authors considers that the basis of 8-Cl-cAMP antiproliferative and apoptotic action is in its biomodulator action [4-7], which involves modulation of the intracellular level of two isoforms of the cAMP-dependent PKA holoenzyme (PKAI and PKAII) kinases [5, 8].

The second group of investigations provided by Gandhi *et al*., [9] and Lamb and Steinberg [10] suggest the effect of 8-Cl-cAMP cannot be explained by its action as a cAMP analogue; rather, tumor-cell cytotoxicity is mediated *via* the product 8-Cl-adenosine (8-Cl-Ado). Data from Taylor and Yeoman [11], Halgren *et al*., [12] and Lamb and Steinberg [10] suggested that 8-Cl-Ado production occurred in the presence of active fetal bovine serum. The second group of authors state that 8-Cl-cAMP is only a pro-drug and that through serum phosphodiesterases and 5’nucleotidases hydrolyzes 8-Cl-cAMP to 8-Cl-adenosine which is now considered the main cytotoxic factor [13]. The experimental data on which the hypothesis of 8-Cl-adnosine is the active principal is based on indirect results. On one side 8-Cl-cAMP apoptotic effect is achieved only if FBS is given in a inactivated form, i.e. that has been previously inactivated by 56$^{o}$C. Heat treatment of FBS inactivates almost all enzymes but not protein degradation or protein denaturation, i.e. 8-Cl-cAMPin the presence of inactivated FBS will not be transformed to 8-Cl-adenosine, and therefore will not induce apoptosis in malignant cells [13].

Mechanistic investigations of 8-Cl-Ado in itself suggested that its cytotoxicity is not mediated through the PKA pathway [8, 14, 15]. Similarly, the growth-inhibitory activity of 8-Cl-Ado is not attributable to the interaction with the classical adenosine receptors [10, 14, 16]. A cAMP independent pathway has been suggested for the action of 8-Cl-Ado that results in a

modulatory effect on PKA-subunit mRNA and protein concentrations in mouse lung epithelial cells. Although the exact mechanism of this down-regulation is not known, an RNA-directed action has been suggested [10, 17]. Taken together, this group of investigations delineate that the role of 8-Cl-cAMP is to generate 8-Cl-Ado, which in turn acts as a nucleoside analogue to exert cytotoxicity. Also, the data demonstrated by Gandhi *et al*., [9] and Lamb and Steinberg [10] show that 8-Cl-cAMP serves as a pro-drug and is converted to 8-Cl-Ado in medium with subsequent phosphorylation to accumulate as 8-Cl-ATP in cells. This is especially important if one considers that the action of 8-Cl-cAMP is attributable to 8-Cl-Ado. This compound resembles a classical nucleoside analogue, which must be converted to its phosphorylated form before incorporation into nucleic acids or actions on other cellular targets. These and other studies suggest differential sensitivity to cytotoxic and gentoxic activity of 8-Cl-cAMP [18-21]. In addition, to further complicate the debate of 8-Cl-cAMP action, a research team headed by Park *et al*., [22] using a cDNA microarray genome wide expression profiling of 8-chloroadenosine and 8-chloro-cAMP in tumor cells showed that 8-Cl-cAMP and 8-Cl-adenosine made differential patterns of gene expression for their anticancer activities, however, sharing some common distal events with each other. Interestingly, even though 8-Cl-adenosine did not share the clusters of genes that where up-regulated, genes related to differentiation and development, 8-Cl-adenosine induced a significant overlap [with 8-Cl-cAMP] of gene clusters that where down regulated, genes related to proliferation and transformation, thereby giving a possible underlying mechanism of its anticancer activity by executing programmed cell death [21, 23]. Relationship between 8-Cl-cAMP and its metabolites has been investigated by using liquid chromatography-HPLC. Cummings *et al*., [24] shown that in the tumor tissue taken by biopsy after a 7day continuous infusion of 8-Cl-cAMP found 8-Cl-adenosine in 100% greater concentrations then expected or 100 times bigger concentrations, more than LC50 which has been defined previously *in vitro* [9, 24, 25]. Still, as we know that 8-Cl-cAMP is hydrolyzed very slowly and that 8-Cl-adenosine is a poor substrate for adenosine deaminase, and that the investigation has been done on only one sample of tumor tissue, the authors discuss that the presence of 8-Cl-cadenosine maybe be a consequence of accumulation during the 7 day period. To further debate on the matter, in the same work in the plasma no metabolite were found, only 8-Cl-cAMP [24]. Still, the theory of 8-Cl-adenosine as the main cytotoxic effectors in cancer cells is still on glass feet, as the results of previous investigations have not been surpassed as the results show biochemical specificity in cells incubated with 8-Cl-adenosine versus 8-Cl-cAMP [25-29]. Based on these results 8-Cl-cAMP may act by a dual mechanism, i.e. Kim *et al*.,[17] showed that the mechanism of 8-Cl-cAMP can be perceived separated, 8-Cl-cAMP and by 8-Cl-adenosine.

**PKA the 8-Cl-cAMP Receptor**

Until the 90's only two groups of intracellular receptors cAMP and cAMP analogues have been defined. In this first group we have PKA I and II, and today's we know another group of effectors which are named guanine exchange factors or GEF or EPAC. We have to types of receptors, cAMP-GEF I and cAMP-GEF II [30, 31]. These factors control connection and release of guanine nucleotide from Rap-1 protein, and therefore interfere with its activity. The Rap 1 protein is in the scope of the ras signal pathway, so cAMP by this mechanism can control cell proliferation and differentiation [31]. One of the best investigated down stream pathway effects of Rap-1 is the control of ERK type MAP kinase activity [32] which is known by is role in inhibiting apoptosis [33]. Still in cells under the influence of 8-Cl-cAMP did not register a significant change in the activity of MAP kinase, and also the usage of a MAP inhibitor did not influence the occurrence of apoptosis by 8-Cl-cAMP [34]. Based on these results the authors have concluded that the main rout of 8-Cl-cAMP activity is by modulation of PKA activity.

Protein kinase A is an enzyme in mammalian, which is present in two isoforms: PKA, PKA II [1,2]. Structurally, these isoforms are composed from catalytic and regulatory subunits (Figure **1**). Every isoform in its composition has two types of regulatory units by which they can differentially distinguished: I α and RI ß, i.e. RI α and RI ß goes into the RI isoform and RII α and RII ß goes into the R II isoform [35]. The occurrence of RI and RII types of regulatory unites differentiate by the phase of the cell cycle, and between cells of different tissues. Still, most abundant are the RI α and RII ß subunits.

The isoforms of PKA can be also differentiated by their affinity towards camp, sub cellular distribution and function. On every regulatory subunit there are two places for cAMP attachment, the attachment place A and the attachment place B [35]. 8-Cl-cAMP as a

specific cAMP analogue is attached on both places (A and B), same as cAMP, but with different affinity [36-38]. With the strongest affinity 8-Cl-cAMP is attached to place B on the RII subunit and for place A the weakest. On the other hand 8-Cl-cAMP has a high affinity towards place A on the RI subunit and moderate affinity for place B. In micro molar concentrations 8-Cl-cAMPis connected only on the B place of the RII subunit, so that PKA II stays in form of a holoenzyme, of which by a negative biofeedback pool brings a decrease in the synthesis of PKA I [1, 2, 17, 39]. The foundation of anti-proliferative activity of 8-Cl-cAMP is based on the decease or downstream regulation of PKAI in malignant transformed cells [17, 39]. The fundamental importance of 8-Cl-cAMP down regulation of PKAI is best understood through the functions of this enzyme in a cancerous cell. By investigating the function of PKA it has been observed that there is a correlation between the level of differentiation and malignant transformation of cells that have different expression patterns of PKA isoforms. An increase expression of PKA II in contrast to PKAI is found in normal, non-proliferative cells and also cell in the interphase. In opposite, in tumor tissues and normal cells, which began there, cell cycle, i.e. in proliferation there is an increase in the expression of PKAI isoform. The physiological role of PKAI is conducting mitogen signals [35]. A continuous high level of PKA expression help cells to proceed through all the phases of the cell cycle even if the cells are incubated by a medium with no growth factors [39]. PKAI participates in the production of angiogen factors. It is a well established fact that 8-Cl-cAMP treated malignant cells reacts in a dose response way by decreasing the level of VEGF and bFGF production, and also the cancer cells have a decreased ability to invade through the matrix a of the basal membrane [10]. The subunit RI alpha is directly connected to cytochrome oxidase C, so a decrease in intracellular levels of PKA I cause a release of cytochrome C and consequently induction of apoptosis which might be one of the mechanisms of 8-Cl-cAMP apoptotic properties [40]. Changes in transcription of the gene for RI subunit are initiated 10 min after exposition of cells to 8-Cl-cAMP [2]. In this process translocation is preceded by PKA II or RII subunit transport to the nucleus. In regard that PKA II is a cytoplasmatic enzyme, its translocation to the nucleus is a prerequisite for its action on gene transcription. In early research nuclear translocation of PKA II is registered through normal development [41], hormone dependent regression of tumors and reversion of transformation. In primary structure RII molecule there is sequence of 4 positively electropositive aminoacides $^{244}$KKRK$^{247}$ which enabled the transport to the nucleus [41]. Even though we have a great structural homology, RI sub unit doesn't posses this sequence of amino acids because this receptor molecule doesn't migrate to the nucleus. It has been established that under the influence of 8-Cl-cAMP the magnitude of translocation of RII subunit protein is 5 to 10 times bigger than by other cAMP analogues [42], which shows us that the level of translocation is correlated with the level of inhibition of proliferation of malignant cells. In the composition of the promoter of the gene which its transcription is regulated by intracellular concentration of cAMP, is the CRE sequence (cAMP responsive element) [43]. Based on these results, 8-Cl-cAMP is to be seen as a molecule that leads into restoration of gene transcription in a malignant cell by increasing the concentration of transcription factors that directly connect to the CRE region, which is preceded by the translocation of the RII protein receptor into the nucleus [42].

Lamb and Steinberg [10] have estimated that on a culture of MCF-7 cells of the malignant breast tumor 8-Cl-cAMP has achieved a decrease in proliferation, but this process has not been influenced by the ratio of PKA I versus PKA II. On the same culture the cholera toxin has established a significant decrease in the ratio of PKA I/PKA II, but at the same time hasn't influenced proliferation, i.e. its inhibition. Based on these results these authors suggested that down stream regulation PKA I by 8-Cl-cAMP is not the mechanism of its anti proliferative action [10].

Little attention has been given to ectogen protein kinase A or ECPKA. This kinase is situated on the outer layer of cell membrane, and immunologically and biochemical is related to intracellular PKA [44-47]. Cyclic AMP, which stimulated ECPKA, is derived from forskolin sensitive intracellular source. Expression of RI and RII subunits ECPKA is determined by intracellular signals that regulate growth. An increased level of RI alpha subunit causes an increase in expression level of ECPKA [44-47]. Still, in cancer patients a higher level of ECPKA has been found in comparison to normal subject [48,49]. For the time being there is only scarce data that 8-Cl-cAMP as a cAMP analogue goes through the cell membrane. What kind of biochemical reaction triggers 8-Cl-cAMP to activate ECPKA, and does this interaction have a role in inhibition of growth; reversal of differentiation or apoptosis in malignant transformed cell still has to be elucidated.

### 8-Cl-cAMP Combinational Therapy

8-Cl-cAMP combinational [50] therapy through *in vitro* exploration, shows that 8-Cl-cAMP achieves LC50 levels after 72h incubation and these results have been achieved in various number of malignant cell lines [50-53]. Still, a number of investigations have been done to stimulate 8-Cl-cAMP to achieve better cytotoxicity of other antitumor agents. 8-Cl-cAMP used in concentrations that hasn't achieved any effects has elevated the cytotoxic activity of cisplatine and paclitaxel on a number of *in vitro* models including cell lines of breast carcinoma, carcinoma of the lungs, melanoma, colorectal and carcinoma of the head and neck. A similar but less effect 8-Cl-cAMP has achieved in the combination with cisplatin [54]. New investigations have tried combination of 8-Cl-cAMP with tiazofurin and sulfinosine [55-57]. With paclitaxel 8-Cl-cAMP has potentate's antitumor activity of paclitaxel on different cultures of human carcinoma of ovaries [58]. Also, very nice expression of antitumor activity towards an *in vivo* melanoma model in C57 mice has been achieved by combination of 8-Cl-cAMP and a taxane, docetaxel [59].

*In vivo* 8-Cl-cAMP as a solo therapy showed a good antitumor effect on the xenographts of colorectal carcinoma, breast, ovarian and pancreas carcinoma [1,3-6]. The rationale for combination therapy comes from the overall knowledge of the mechanisms from individual action of these substances, i.e. 8-Cl-cAMP and taxanes in which a crucial role in synergistic action plays PKA.

The activity of PKA II is based on a dependent relationship with anchoring proteins or AKAP. Anchoring proteins are controllably expressed on the membranes of organelles. So only when PKA II is attached to them a opening is placed for cAMP. This way the expression of PKA II is expressed by the concentration of cAMP in the vicinity of this environment. One of the cloned anchoring proteins is expressed at the centromeres in eukaryote cells, so alteration of normal functioning of tubulin polymers conditions the activation of the PKA II enzyme. This way activated PKA II phosphorylates bcl-2 protein which is defined as one of crucial factors in the proapoptotic action of antitumor agents with antitubuline activity, including taxanes [60-62]. One the other side it was thought that PKA I enzyme was free in the cytoplasm, so that the regulation of its activity is solely based on the concentration of cAMP in subcellular region. PKA I is concentrated and connected to the microtubules in all phases of the cell cycle, and this enzyme has multiple roles in the regulation of the dynamic instability of microtubules and/or there regulatory complexes during the process of mitosis [63]. At the same time, it has been showed that the activation of PKA I leads to phosphorylation and partial degradation of stathmines [64]. Stathmines are regulators of micrtubule dynamics and exert there activity in contrast to the stability activity of MAP, so that statmine phosphorylation tends to enhance the process of tubule polymeraization [65]. Because of its greater affinity towards PKA, 8-Cl-cAMP brings a massive activation of this enzyme. The release of the catalytic subunits from 8-Cl-cAMP phosphorylates stathmin which brings an increase in tubulin polymerization. This situation is ideal for taxanes because these agents only react to polymerized tubuline. Except stathmin, PKA I phosphorylates raf-1 [60] which is another route to stimulate the group of taxanes. On the other hand, the compromised activity of microtubules activates enzyme PKA II which is located on the centromeres with a consequence of bcl-2 phosphorylation.

## CONCLUSION

Clinical experience of 8-Cl-cAMP has been a result from three phases of clinical studies I, where a defined maximum tolerated dose has been defined MTD and dose limit toxicity DLT [66-68]. From the registered unwanted negative adverse effects of 8-Cl-cAMP the only important results is the finding of a increase in serum Ca (free) and consequently nephrotoxicity which can be readily regulated by supportive therapy. This profile of 8-Cl-cAMP is especially beneficial for combinational therapies with other cytotoxic agents, as it does not increase their myelotoxic effects that is one of the main problems of combinational therapy in oncology. Still, 25 years later cAMP analogues are present in a state of ambiguity, one is that they haven't found there way to therapy and the other is that there promising properties still accelerate researchers to further investigate this biological tool for the investigation of cell proliferation and differentiation thus broadening and bearing the idea of biomulation to the cancer research community.

## ACKNOWLEDGMENTS

The research has been funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, project No. OI 173034.

## REFERENCES


[1] Yoon S, Cho-Chung. Role of Cyclic AMP Receptor proteins in growth, differentiation and suppression of malignancy: new approaches to therapy. Cancer Research 1990; (50): 7093-7100.

[2] Oliveira PF, Martins AD, Moreira AC, Cheng CY, Alves MG. The Warburg effect revisited-lesson from the Sertoli cell. Med Res Rev 2015; 35(1): 126-51. http://dx.doi.org/10.1002/med.21325

[3] Lucchi S, Calebiro D, de Filippis T, Grassi ES, Orietta Borghi M, Persani L. 8-Chloro-Cyclic AMP and protein kinase A I-selective cyclic AMP analogs inhibit cancer cell growth through different mechanisms. PLoS One 2011; (6)6: e20785. http://dx.doi.org/10.1371/journal.pone.0020785

[4] Dicitore A, Grassi ES, Caraglia M, Borghi MO, Gaudenzi G, Hofland LJ, Persani L, Vitale G. The cAMP analogs have potent anti-proliferative effects on medullary thyroid cancer cell lines. Endocrine 2015; 1-12. http://dx.doi.org/10.1007/s12020-015-0597-7

[5] Ramage AD, Langdon SP, Ritchie AA, Burns DJ, Miller WR. Growth inhibition by 8-chloro cyclic AMP of human HT29 colorectal and ZR-75-1 breast carcinoma xenografts is associated with selective modulation of protein kinase A isoenzymes. Eur J Cancer 1995; 31(6): 969-73. http://dx.doi.org/10.1016/0959-8049(95)00190-5

[6] Scala S, Budillon A, Zhan Z, Cho-Chung YS, Jefferson J, Tsokos M, Bates SE. Downregulation of mdr-1 expression by 8-Cl-cAMP in multidrug resistant MCF-7 human breast cancer cells. J Clin Invest 1995; 96(2): 1026-34. http://dx.doi.org/10.1172/JCI118088

[7] Ciardiello F, Tortora G, Kim N, Clair T, Ally DS. 8-Chloro-cAMP inhibits transforming growth factor alfa transformation of mammary epithelial cells by restoration of the normal mRNA patterns for cAMP- dependent protein kinase regulatory subunit isoforms wich disruption upon transformation. J Biol Chem 1990; 265: 1016-1020.

[8] Ally S, Tortora G, Cair T, *et al.* Selective modulation of protein kinase isozymes by selective 8-Cl-cAMP provides a biological means for control of human colon cancer cell growth. Proc Natl Acad Sci USA 1988; 85: 6319-6322. http://dx.doi.org/10.1073/pnas.85.17.6319

[9] Gandhi V, Ayres M, Halgren RG, Krett NL, Newman RA, Rosen ST. 8-chloro-cAMP and 8-chloro-adenosine act by the same mechanism in multiple myeloma cells. Cancer Res 2001; 61: 5474-5499.

[10] Lamb D, Steinberg RA. Anti-proliferative effects of 8-chloro-cAMP and other cAMP analogs are unrelated to their effects on protein kinase A regulatory subunit expression. J Cell Physiol 2002; 192: 216-224. http://dx.doi.org/10.1002/jcp.10131

[11] Taylor CW, Yeoman LC. Inhibition of colon tumor cell growth by 8-chloro-cAMP is dependent upon its conversion to 8-chloro-adenosine. Anticancer Drugs1992; 3: 485-491. http://dx.doi.org/10.1097/00001813-199210000-00007

[12] Halgren RG, Traynor AE, Pillay S, Zell JL, Heller KF, Krett NL, Rosen ST. 8Cl-cAMP cytotoxicity in both steroid sensitive and insensitive multiple myeloma cell lines is mediated by 8Cl-adenosine. Blood 1998; 92: 2893-2898.

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

[14] Tortora G, Ciardiello F. Targeting of epidermal growth factor receptor and protein kinase A: molecular basis and therapeutic applications. Ann Oncol 2000; 11: 777-783. http://dx.doi.org/10.1023/A:1008390206250

[15] Gandhi V, Ayres M, Halgren RG, Krett NL, Newman RA, Rosen ST. 8-chloro-cAMP and 8-chloro-adenosine act by the same mechanism in multiple myeloma cells, Cancer Res 2001; 61: 5474-5499.

[16] Lange-Carter CA, Vuillequez JJ, Malkinson AM. 8-Chloroadenosine mediates 8-chloro-cyclic AMP-induced down-regulation of cyclic AMP-dependent protein kinase in normal and neoplastic mouse lung epithelial cells by a cyclic AMP-independent mechanism. Cancer Res 1993; 53: 393-400.

[17] Kim SN, Kim SG, Park JH, Lee MA, Park SD, Cho-Chung YS, Hong SH. Dual anticancer activity of 8-Cl-cAMP: inhibition of cell proliferation and induction of apoptotic cell death. Biochem Biophys Res Commun 2000; 273: 404-410. http://dx.doi.org/10.1006/bbrc.2000.2949

[18] Boe R, Gjertsen BT, Doskeland S, Vintermyr OK. 8-Chloro-cAMP induces apoptotic cell death in a human mammary carcinoma cell (MCF-7) line. Br J Cancer 1995; 72: 1151-1159. http://dx.doi.org/10.1038/bjc.1995.479

[19] Pesic M, Podolski A, Rakic L, Ruzdijic S. Purine analogues sensitize the multidrug resistant cell line (NCI-H460/R) to doxorubicin and stimulate the cell growth inhibitory effect of verapamil. Inves New Drugs 2010; 28(4): 482-92. http://dx.doi.org/10.1007/s10637-009-9277-x

[20] Bajic V, Stanimirovic Z, Stevanovic J, Spremo-Potparavic B, Zivkovic L, Milicevic Z. Cytogenetic effects of 8-Cl-cAMP on human and animal chromosomes. Journal of BUON 2009; 14: 71-77. http://dx.doi.org/10.1016/j.arcmed.2004.01.004

[21] Bajic V, Spremo-Potparevic B, Stanimirovic Z, Stevanovic J. Genotoxicity Potential of 8-Cl- Cyclic Adenosine Monophosphate Assessed with Cytogenetic test *in vivo.* Arch Med Res 2004; 35: 209-214.

[22] Park GH, Choe J, Choo HJ, Park YG, Sohn J, Kim MK. Genome-wide expression profiling of 8-chloroadenosine- and 8-chloro-cAMP-treated human neuroblastoma cells using radioactive human cDNA microarray. Exp Mol Med 2002; 34: 184-193. http://dx.doi.org/10.1038/emm.2002.27

[23] Bradbury AW, Carter DC, Miller WR, Cho-Chung YS, Clair T. Protein kinase. A (PK-A) regulatory subunit expression in colorectal cancer and relted mucosa. Br J Cancer 1994; 69: 738. http://dx.doi.org/10.1038/bjc.1994.139

[24] Cummings J, Langdon SP, Ritchie AA, Burns DJ, Mackay J, Stockman P, Leonard RC, Miller WR. Pharmacokinetics, metabolism and tumour disposition of 8-chloroadenosine 3', 5'-monophosphate in breast cancer patients and xenograft bearing mice. Ann Oncol 1996; 7: 291-6. http://dx.doi.org/10.1093/oxfordjournals.annonc.a010574

[25] Taylor CW, Yeoman LC. Inhibition of colon tumor cell growth by 8-chloro-cAMP is depedent upon its conversion to 8-chloro-adenosine. Anticancer Drugs 1992; 5: 485-491. http://dx.doi.org/10.1097/00001813-199210000-00007

[26] Han Z, Chattrjee D, Wyche J. Proliferation of nontrasformed cells is inhibited by adenosine metabolite of but not by parental 8-Cl-cyclic AMP. J Pharmacol Exp Ther 1993; 265: 790-794.

[27] Lang-Carter CA, Vuilleques JJ, Malkinson AM. 8-Chloro-adenosine mediates 8-chloro-cyclic AMP-induced down-regulation of cyclic AMP-dependent proteinkinase in normal and neoplastic mouse lung epithelial cells by a cyclic AMP indepedent mechanism. Cancer Res 1993; 53: 393-400.

[28] Langveld CH, Jongenelen CA, Theeuwes JW, *et al.* The antiproliferative effect of 8-chloro-adenosine, an active metabolite of 8-chloro-cyclic adenosine monophosphate, and disturbances in nucleic acid synthesis and cell cycle kinetics. Biochem Pharmacol 1997; 53: 141-148. http://dx.doi.org/10.1016/S0006-2952(96)00593-X

[29] Rohlff C, Glazer R. Regulation of multidrug resistance though the cAMP and EGF signalling pathways. Cell signal 1995; 7: 431-443. http://dx.doi.org/10.1016/0898-6568(95)00018-K

[30] De Rooij J, Zwartkruis FJ, Verheijen MH, Cool RH, Nijman SM, Wittinghofer A, Bos JL. Epac is a Rap1 guanin nucleotide exchange factor directly activated by cyclic AMP. Nature 1998; 396: 474-477. http://dx.doi.org/10.1038/24884

[31] Kawasaki H, Springett GM, Mochizuki N, Toki S, Nakaya M, Matsuda M, Housman DE, Graybiel AM. A family of cAMP binding proteins that directly activate Rap1. Science 1998; 282: 2275. http://dx.doi.org/10.1126/science.282.5397.2275

[32] Vossler MR, Yao H, York RD, Pan MG, Rim CS, Stork PJ. cAMP activates MAP kinase and Elk-1 through a B-Raf- and Rap1-dependent pathway. Cell 1997; 89(1): 73-82. http://dx.doi.org/10.1016/S0092-8674(00)80184-1

[33] Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science 1995; 270: 1326. http://dx.doi.org/10.1126/science.270.5240.1326

[34] Kim SN, Ahn YH, Kim SG, Park SD, Cho Chung YS, Hong SH. 8-Cl-cAMP induces cell cycle specific apoptosis in human cancer cells. Int J Cancer 2001; 93: 33. http://dx.doi.org/10.1002/ijc.1308

[35] Tortora G, Ciardiello F. Protein Kinase A Type I: A Target For Cancer Therapy. Clin Cancer Res 2002; 8: 303-304.

[36] Cho-Chung YS, Clair T, Tagliaferri P, *et al*. Basic science review: Site-selective cyclic AMP analogs as new biological tool in growth differentiation and proto-oncogene regulation. Cancer Invest 1989; 7: 161-177. http://dx.doi.org/10.3109/07357908909038282

[37] Choi KY, Cho YJ, Kim JS, Ahn YH, Hong SH. SHC1 sensitizes cancer cells to the 8-Cl-cAMP treatment. Biochem Biophys Res Commun 2015; 463(4): 673-8. http://dx.doi.org/10.1016/j.bbrc.2015.05.123

[38] Ahn YH, Jung JM, Hong SH. 8-Cl-cAMP and its metabolite, 8-Cl-adenosine induce growth inhibition in mouse fibroblast DT cells through the same pathways: protein kinase C activation and cyclin B down-regulation. J Cell Physiol 2004; 201(2): 277-85. http://dx.doi.org/10.1002/jcp.20047

[39] Tortora G, Pepe S, Bianco C, Baldassarre G, Budillon A, Clair T, Cho-Chung YS, Bianco AR, Ciardiello F. The RI alpha subunit of protein kinase A controls serum dependency and entry into cell cycle of human mammary epithelial cells. Oncogene 1994; 9(11): 3233-40.

[40] Yang WL, Iacono L, Tang WM, Chin KV. Novel function of the regulatory subunit of protein kinase A: regulation of cytochrome c oxidase activity and cytochrome c release. Biochemistry 1998; 37(40): 14175-80. http://dx.doi.org/10.1021/bi981402a

[41] Jungmann RA, Kranias EG. Nuclear phosphoprtoein kinases and the regulation of gene transcription. Int J Biochem 1997; 8: 819. http://dx.doi.org/10.1016/0020-711X(77)90049-0

[42] Mednieks MI, Yokozaki H, Merlo GR, Tortora G, Clair T, Ally S, Tahara E, Cho-Chung YS. Site-selective 8-Cl-cAMP which causes growth inhibition and differentiation increases DNA (CRE)-binding activity in cancer cells. FEBS Lett 1989; 254(1-2): 83-8. http://dx.doi.org/10.1016/0014-5793(89)81014-2

[43] Roesler WJ, Vadenbark GR, Hanson RW. Cyclic AMP and the induction of eukaryotic gene transcription. J Biol Chem 1988; 263: 9063.

[44] Hatmi M, Gavaret JM, Elalamy I, Vargafig BB, Jacquemin C. Evidence for cAMP-depedent platelet ectoproteinkinase activity that phosphorylates platelet glycoprotein IV (CD36). J Bio Chem 1996; 271: 24776. http://dx.doi.org/10.1074/jbc.271.40.24776

[45] Kang ES, Gates RE, Chiang TM, Kang AH. Ectoprotein kinase activity of the isolated rat adipocite.Bioch Biophys Res Commun 1979; 86: 769. http://dx.doi.org/10.1016/0006-291X(79)91779-0

[46] Kubler D, Pyerin W, Bill O, Hotz, Sonka J, Kinzel V. Evidence for ectoprotein kinase activity that phosphorylates Kemptide in a cyclic AMP-depedent mode. J Biol Chem 1992; 261: 14549.

[47] Schaeger E, Kohler G. External cyclic AMP-depedent protein kinase activity in rat C-6 glioma cell. Nature 1976; 260: 705. http://dx.doi.org/10.1038/260705b0

[48] Cvijic ME, Kita T, Shin W, Dipaola RS, Chin KV. Extracullar catalytic subunit activity of the cAMP depedent protein kinase in prostate cancer. Clin Cancer Res 2000; 6: 2309.

[49] Wang H, Li M, Lin W, *et al*. Extracellular activity of cyclic AMP-dependent protein kinase as a biomarker for human cancer detection: distribution characteristics in a normal population and cancer patients. Cancer Epidemiol Biomarkers Prev 2007; 16(4): 789-95. http://dx.doi.org/10.1158/1055-9965.EPI-06-0367

[50] Pepe S, Tortora G, Noguchi PD, Marti GE, Washington GC, Cho-Chung YS. Effects of 8-chloroadenosine 3',5'-monophosphate and N6-benzyl-cyclic adenosine 5'-monophosphate on cell cycle kinetics of HL-60 leukemia cells. Cancer Rese 51: 6263-7.

[51] Szekeres T, Cho Chung YS, Weber G. Action of Tiazofurin and 8-Cl-cAMP in human colon and pancreatic cancer cells. Cancer Biochem Biophys 1992; 13: 67.

[52] Tagliaferri P, Katsaros D, Clair T, *et al*. Synergistic inhibition of growth of breast and colon human cancer cell lines by site-selective cyclic AMP analogues. Cancer Res 1988; 48(6): 1642-50.

[53] Takanashi A, Yasui W, Yoshida K, Yokozaki H, Saito D, Abe K, Urakami K, Miki K, Tahara E. Inhibitory effect of 8-chloro-cyclic adenosine 3',5'-monophosphate on cell growth of gastric carcinoma cell lines. Jpn J Cancer Res 1991; 82(3): 325-31. http://dx.doi.org/10.1111/j.1349-7006.1991.tb01849.x

[54] Tortora G, di Isernia G, Sandomenico C, Bianco R, Pomatico G, Pepe S, Bianco AR, Ciardiello F. Synergistic inhibition of growth and induction of apoptosis by 8-chloro-cAMP and paclitaxel or cisplatin in human cancer cells. Cancer Res 1997; 57(22): 5107-11.

[55] Janković D, Pesić M, Marković J, Kanazir S, Marković I, Rakić Lj, Ruždijić S. The combination of sulfinosine and 8-Cl-cAMP induces synergistic cell growth inhibition of the human neuroblastoma cell line *in vitro*. Invest New Drug 2006; 24(1): 15-25. http://dx.doi.org/10.1007/s10637-005-4539-8

[56] Korićanac LB, Todorović DV, Popović NM, Demajo MA, Ruzdijić SD, Ristić-Fira AM. Inhibition of B16 mouse melanoma cell growth and induction of apoptotic cell death with 8-chloroadenosine-3',5'-monophosphate and tiazofurin. Ann N Y Acad Sci 2004; 1030: 384-92. http://dx.doi.org/10.1196/annals.1329.048

[57] Drabek K, Pesić M, Piperski V, Ruzdijić S, Medić-Mijacević L, Pietrzkowski Z, Rakić L. 8-Cl-cAMP and tiazofurin affect vascular endothelial growth factor production and glial fibrillary acidic protein expression in human glioblastoma cells. Anti-Cancer Drug 2000; 11(9): 765-70. http://dx.doi.org/10.1097/00001813-200010000-00014

[58] McDaid HM, Johnston PJ. Synergistic interaction between paclitaxel and 8-chloro-adenosine 3',5'-monophosphate in human ovarian carcinoma cell lines. Clin Cancer Res 1999; 5: 215-20.

[59] Bjelogrlic S, Koricanac A. Dissertation: Investigation of cytotoxic and antitumor activity of Taxane group of compounds in combination with a structural analogue cyclic adenosine 3', 5' monophosphate on the mouse model of melanoma *in vivo* and *in vitro*. Medical Faculty, University of Belgrade, Serbia 2004.

[60] Blagosklonny MV, Giannakakou P, Wafik SD, Kingston GI, Higgs PI, Fojo T. Raf-1/bcl-2 Phosphorylation: A step from microtubule damage to cell death. Cancer Res 1997; 57: 130-135.

[61] Haldar S, Jena NI, Groce CM. Inactivation of bcl-2 by phosphorylation. Proc Natl Acad 1995; 89: 5768-72. http://dx.doi.org/10.1073/pnas.92.10.4507

[62] Srivastava RK, Srivastava AR, Korsmeyer SJ, *et al.* Involvement of microtubules in the regulation of Bcl2 phosphorylation and apoptosis through cyclic AMP-dependent protein kinase. Mol Cell Biol 1988; 18: 3509-3515. http://dx.doi.org/10.1128/MCB.18.6.3509

[63] Imaizumi-Scherre T, Faust DM, Barradeau S, Hellio R, Weiss MC. Type I protein kinase a is localized to interphase microtubules and strongly associated with the mitotic spindle. Exp Cell Res 2001; 264: 250. http://dx.doi.org/10.1006/excr.2001.5164

[64] Gradin HM, Larsson N, Marklund U, Gullberg M. Regulation of microtubil dynamics by extracellular siganls: cAMP-depedent protein kinase switches of the activity of oncoprotein 18 in intact cells. J Cell Biol 1998; 140: 131. http://dx.doi.org/10.1083/jcb.140.1.131

[65] Horwitz SB, Shen HJ, He L, Dittmar P, Neef R, Chen J, Schubart UK. The microtubule-destabilizing activity of metablastin (p19) is controlled by phosphorylation. J Biol Chem 1997; 272: 8129. http://dx.doi.org/10.1074/jbc.272.13.8129

[66] Saunders MP, Salisbury AJ, Harris AL, Long L, O'Byrne KJ, Macaulay VM, Miki K, Cho-Chung YS, Talbot DC. Phase I study of the protein kinase A regulator 8-chloro cAMP. Proc Am Assoc Cancer Res 1995; 36: 241.

[67] Tortora G, Ciardiello F, Pepe S, Tagliaferri P, Ruggiero A, Bianco C, Guarrasi R, Miki K, Bianco AR. Phase I clinical study with 8-Cl-cAMP and evaluation of immunological effects in cancer patients. Clin Cancer Res1995; 1: 377-384.

[68] Propper DJ, Saunders MP, Salisbury AJ, *et al.* Phase I study of the novel cyclic AMP cAMP analogue 8-chloro-cAMP in patients with cancer: toxicity, hormonal, and immunological effects. Clin Cancer Res 1999; 5: 1682-9.

Received on 05-10-2015 Accepted on 12-10-2015 Published on 26-11-2015

DOI: http://dx.doi.org/10.6000/1929-2279.2015.04.04.5