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The influence of 8-Cl-cAMP on tumor marker CA19.9 on experimental glioma in rats

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BACKGROUND: *Since the existing chemotherapeutics can not control all neoplastic processes, there is a need for discovering of new, more efficient drugs for cancer treatment and developing of specific tumor markers which would enable earlier diagnosis and follow up of cancer. The aim of this paper was to investigate the influence of 8-Cl-cAMP on expression of CA19.9 tumor marker in C6 cell line, impalnted in Wistar rat's brain.*

METHODS: *Determination of CA 19.9 concentration in sera and brain tissue supernatants has been performed by immunoenzyme assay. Experimental glioma was induced by injection of $4x10^9$ C6 cells into Wistar rat's brain. 8-Cl-cAMP was administered intraperitoneally in a single (50mg/kg/day) and multiple doses (50mg/kg/day, 5 days).*

RESULTS: *The referent values of CA 19.9 in brain tissue supernatant ranged from 2.63 to 10.83 U/ml/ The rate of concentrations of CA 19.9 was determined at five time points (1, 7, 14, 21 and 31 day) after the tumor cell implantation. Application of multiple doses of 8-Cl-cAMP significantly decreased CA 19.9 concentration, by 84.03 percent, at the seventh day of post treatment.*

CONCLUSION: *Obtained results suggested that CA 19.9 might be used as a tumor marker for determination of 8-Cl-cAMP influence on experimental glioma in rats.*

KEY WORDS: *Cyclic AMP; Glioma; Tumor Markers, Biological; CA-19-9 Antigen*

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INTRODUCTION

In spite of fact that in the last twenty years a great progress in cancer treatment was achieved, the treatments are not equally efficient for different types of tumors. Solid tumors and metastasis are still resistant to therapy and among them, brain tumors represent a specific problem (1).

The pharmacological approach in the synthesis of novel drugs suggests the use of purine nucleoside analogs in which heterocyclic structure or sugar moiety is altered in such a way that causes toxic effect when they incorporate in different part of cell. Various compounds used for chemotherapy differ in their chemical structure and mechanism of action as well (2). Widely used nucleoside analogs have substituents on purine and pirimidine ring, which do not exist in natural form. They change the pattern of base pairing or interaction of nucleotide with specific enzymes. 8-Chloro-cyclic adenosine 3'5'monophosphate (8-Cl-cAMP, NSC 284751) belongs to C8 analog group. Neither of, so far examined C2, C6 or C8 analog of cAMP, showed such a great regulatory effects on the growth inhibition of wide spectrum of human cancerous cell lines, as 8-Cl-cAMP did (3).

The mechanisms of normal differentiation in cancer cells are disturbed, but could be restored by the action of 3'5'monophosphate analog through specific receptors (4). Cyclic AMP, as the intracellular regulating factor, plays a role in differentiation and growth regulation in different cell types; however, the exact mechanism of action is still unknown (5). In mammalian cells cAMP is activated via cAMP dependent protein kinase (PKA) receptors PKA I and PKA II (6,7).

The mechanism of action of 8-Cl-cAMP is still unknown. It is supposed that inhibitory growth effect of 8-Cl-cAMP might be in relation with decreased ratio of PKA I / PKA II isoforms in cancer cells (8). However, some results suggest that inhibitory growth effect of 8-Cl-cAMP could be mediated by its metabolite 8-Cl- adenozine

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as well (9).

Oncofetal antigens play important role in diagnosis and /or follow up of malignant tumors. Among them widely known are carcinoembrionic antigen (CEA) and α-fetoprotein (AFP), produced by tumor cells (10). Oncofetal antigens, CEA and AFP, were not found on glioma cells in vitro (11). Tumors also produce a lot of different substances, mostly proteins that are formed as secondary products during malignant process e.g. gastrointestinal carcinoma´s antigen CA 19.9. The CA 19.9 was identified in 1981. The function of this tumor marker is still unknown. The CA 19.9 can be found in tissue as a monosialoganglioside, and in the sera as a high molecular carbohydrate-rich glycoprotein, known as mucin (12-14).

By discovering of a relevant tumor marker whose concentration changes indicate increase or reduction of a tumor burden, one could achieve a better follow up of purine nucleosides, used in chemotherapy of brain tumors.

The aims of this study were: to identify the presence of non specific tumor marker CA 19.9 and to determinate its referent values in rats´ sera and brain tissue supernatants; to determine concentrations of CA 19.9 in sera and rat brain tumor supernatants during tumorigenesis; to evaluate single and multiple doses of 8-Cl-cAMP on concentration of CA 19.9 in rat experimental brain tumor.

MATERIALS AND METHODS

Induction of brain tumor

C6 glioma cells, used for the induction of experimental brain tumor, were generously gifted to us by Dr. Grivenikov from the Institute of molecular genetics, Russian Academy of Science, Moscow.

C6 cells were grown in Dulbecco's modified Eagle's medium (DMEM, ICN Pharmaceuticals) supplemented with 10% of fetal calf serum (FCS, ICN Pharmaceuticals) and antibiotics: 100 IU/ml of penicillin, 100μg/ml of streptomycin, and 25μg/ml of amphoterycin. Cells were cultivated in flasks (Costar) at 37°C in the atmosphere of 100% humidity and 5% of CO_2 (Heraues). Experimental glioma was induced by injection of $4x10^9$ C6 cells into right hemisphere of Wistar rat's brain (15).

Application of 8-Cl-cAMP and determination of CA 19.9

8-Cl-cAMP (ICN Pharmaceuticals) was administered to Wistar rats intraperitoneally (i.p.) in a single (50 mg/kg/day) or multiple doses (50 mg/kg/day, 5 days). Four hours after administration of a single dose and five days after multiple doses of 8-Cl-cAMP, the rats were sacrificed. The concentrations of CA 19.9 tumor marker (U/ml), in sera and brain tissue supernatants, were determined by immunoenzyme assay (Imx, Abbott). Results are evaluated by Student's t-test.

RESULTS

Because CA 19.9 tumor marker has not been evaluated in sera and Wistar rat brain tissue so far, we determined its concentration for the first time in the control group of rats. The control group was composed of: six samples of fresh sera, five samples of sera leftover 24 hours at 4°C, eight samples of fresh brain tissue supernatants, five samples of frozen brain tissue supernatants, and six samples of culture medium (Table 1).

The CA 19.9 tumor marker was not detected in the medium and in the frozen sera, but very low concentrations were found in some fresh sera samples. However, a range of concentrations was measured in the samples of brain tissue supernatants (Table 1).

Table 1. The concentration of CA 19.9 (U/ml) tumor marker in control group

CONTROL GROUP					
SERA		BRAIN TISSUE SUPERNATANT		CULTURE MEDIUM	
Fresh	24h / + 4°C	Fresh	Frozen	Without cells	With cells
0	0	9.04	0	0	0
0	0	7.68	2.48	0	0
0.23	0	7.27	0.57	0	0
0.23	0	5.33	1.69		
0	0	9.52	2.25		
0.18		6.39			
		3.08			
		5.78			

In fresh brain tissue supernatants referent values were between 2.63 and 10.83 U/ml, while in frozen brain tissue samples they were much lower (0.05 - 3.45 U/ml) (Table 2).

Table 2. The refferent values of CA 19.9 in fresh and frozen brain tissue supernatants

	SUPERNATANT OF BRAIN TISSUE	
	FRESH	FROZEN
Total number (N)	8	5
Mean value (x)	6.73	1.75
Minimal value (Min)	3.08	0.57
Maximal value (Max)	9.29	2.48
Standard deviation (SD)	2.05	0.85
Referent values (RV)	2.63 – 10.83	0.05 – 3.45

The rate of CA 19.9 concentrations were determined in five time points i.e. at 1 st, 7th, 14th, 21st, and 31st day after C6 glioma cells implantation.

At the first day of tumorigenesis the mean value of CA 19.9 was 6.7 U/ml, and seven days later its concentration significantly increased (45.2 U/ml) compared to control values ($p<0.001$).

The mean concentration of CA19.9 increased during tumorigenesis reaching its highest level of 71.3 U/ml at the end of the experiment (Figure 1).

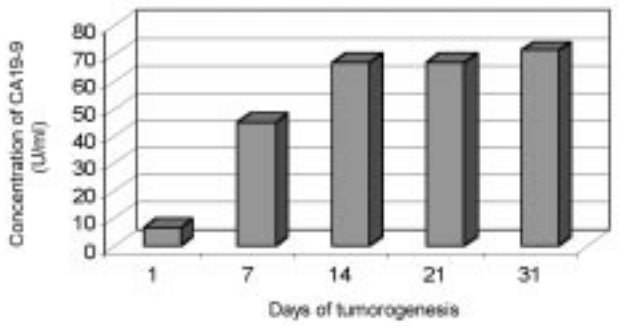

Figure 1. The CA 19.9 tumor marker concentration after C6 glioma cells implantation

8-Cl-cAMP was administered i.p. in single and multiple doses in rats with implanted glioma. The effect of single dose of 8-Cl-cAMP was as follows: four hours after i.p. application of the agent, the concentration of CA 19.9 was not significantly changed compared to untreated rats; seven and fourteen days after treatment, the concentration of CA 19.9 decreased by 42.6% and 47.61% respectively (Figure 2).

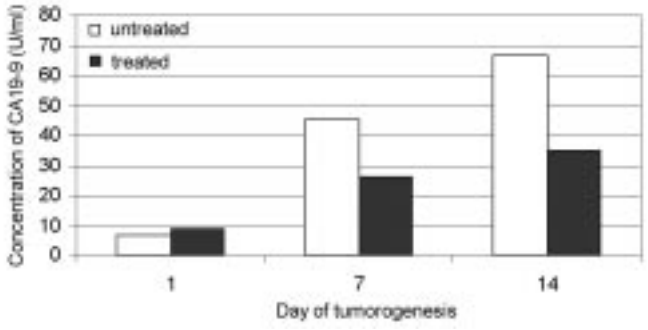

Figure 2. The effect of single dose of 8-Cl-cAMP

The multiple doses of 8-Cl-cAMP induced further decline of the CA 19.9 concentrations. The most prominent decrease (84.03%) was found on 7th day after i.p. application of 8-Cl-cAMP (Figure 3).

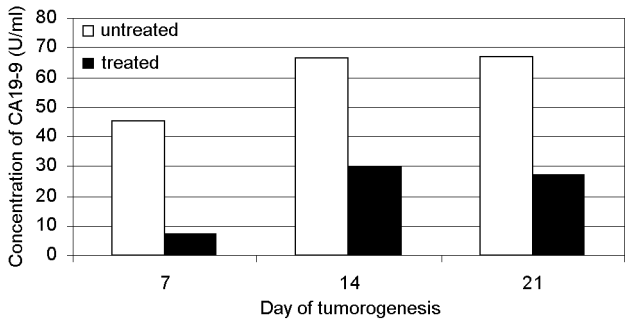

Figure 3. The effect of multiple doses of 8-Cl-cAMP

DISCUSSION

It is well known that tumor cells synthetize and secrete substances, called tumor markers, and their increased concentrations suggest malignancy in organism. CA 19.9 has not been used as marker in diagnosis of brain tumor so far. Before determination of CA 19.9 in biological material its concentration was determined in the culture medium (DMEM) with or without C6 cells (Table 1). Because CA 19.9 has not been evaluated so far in Wistar rats' sera or brain tissue we determined its concentration in control samples. In the sera, concentration of CA 19.9 was very low or undetectable (Table 1). It is supposed that the absence of CA 19.9 in the medium and rat sera is due to lack of secretion by cells. Each sample of brain tissue supernatant contained CA 19.9 that enabled determination of referent values. In fresh brain tissue supernatants referent concentrations of CA 19.9 were significantly higher comapred to frozen brain tissue samples ($p<0.05$) and therefore only fresh brain tissue samples were used throughout the experiment. In supernatants prepared from brain tumor tissue increased CA 19.9 concentrations were found. It is supposed that CA 19.9 is synthetized by C6 glioma cells. After determination of referent values of CA 19.9, its concentration was determined during tumorigenesis, in Wistar rats implanted with C6 glioma cells. Tumorigenesis was followed during 31 days. In other investigations the same model was used in which only a progress of tumor burden was followed in various period after C6 cell implantation (16,17).

The CA 19.9 concentrations were determined in supernatants of brain tumor tissue in five points of time during tumorigenesis (Figure 1). According to obtained results the highest concentration of this tumor marker was found at the seventh day of tumorigenesis. Because of increased concentrations of CA 19.9 (from the first to the seventh day the concentration increased 6.7 times) we presumed that this tumor might have high rate of malignancy and very high growth fraction. The increase of tumor marker concentration during intensive tumor cell division indicates that cells of experimentally induced brain tumor produce this tumor marker. Probably, CA 19.9 tumor marker expresses itself inside the tumor cells. One of the aims of this paper was to investigate the influence of a single and multiple doses of 8-Cl-cAMP through expression of CA 19.9 tumor marker. After intracerebral implantation of C6 glioma cells in rats, a single dose of 8-Cl-cAMP was administered i.p. The CA 19.9 concentration was determined 4 hours later and 7 and 14 days respectively (Figure 2). Four hours after single dose of 8-Cl-cAMP, CA 19.9 concentration was very similar to the concentration obtained in tumor bearing but untreated rats. Seven and fourteen days after i.p. administration of the drug, CA 19.9 concentration decreased by 42.6 % and 47.6% respectively.

After the treatment, using multiple doses of 8-Cl-cAMP during five consecutive days, CA 19.9 concentration was also decreased: the most profound decline was noticed seven days after the treatment i.e. CA 19.9 concentration decreased by 84.03% in com-

parison to untreated animals.

It was obvious that both treatment schedules induced decline of CA19.9 concentrations in brain tumor tissue supernatants. Results obtained suggested that both dose and activity of the 8-Cl-cAMP during treatment were sufficiently high. At the seventh day of tumorigenesis, multiple dose treatment decreased CA 19.9 concentration to the range of referent values. Our results indicated that the effect of 8-Cl-cAMP in the same protocol induced less profound decrease of CA 19.9 concentration probably due to increased tumor burden.

The effect of the 8-Cl-cAMP on human gliomas in *in vitro* studies were evaluated as well. Significant antiproliferative, cytotoxic and differentiation potential of the drug was found (18).

CONCLUSION

Results showed that C6 glioma in rats produced increasing concentration of CA 19,9 tumor marker. Treatment with 8-Cl-cAMP decreased CA 19.9 concentration in tumor brain tissue supernatants. It is suggested that CA 19.9 tumor marker might be used as a tumor marker for the evaluation of 8-Cl-cAMP activity on experimental glioma in rats.

A significance of this finding in relation to clinical studies has to be evaluated.

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