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BILATERAL LESIONS OF THE CAUDATE NUCLEI AND EFFECTS OF THE PSYCHOTONIC DRUG-PIRACETAM ON CANCER DEVELOPMENT IN METHYLCHOLANTHRENE INDUCED TUMORS IN THE RAT

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The theory about the role of central nervous system (CNS) monoamines in cancerogenesis has been debated for decades. The present investigation was designed to examine the effect of bilateral lesions of the caudate nuclei (as an important monoamine structure in the CNS), the effects of a psychotonic drug - Piracetam and an antineoplastic drug, cyclophosphamide, on survival time and incidence of metastases of tumor-bearing rats. We used 102 Wistar rats in the experiment. The tumors were induced by 3-methylcholanthrene. After surgical extirpation of the tumors, different groups of animals were treated with bilateral lesions of the caudate nuclei, the psychoactive drug - piracetam, cyclophosphamide or nothing. Autopsy and histological examinations were performed in all animals. From the group of animals which was treated with bilateral lesions of the caudate nuclei 12,5% survived over 120 days versus 81,2% of the animals from the piracetam group, 68,8% from the cyclophosphamide group and 50% of the control group (only surgical removal of tumors). In the group of animals which was treated with bilateral lesions of the caudate nuclei all of the animals had metastases, whereas in the piracetam group no animals had metastases. In the cyclophosphamide group 45,4% of the animals were without metastases and in control group 27,3% of the animals were without metastases.

The mechanism of the antineoplastic effect monoamine stimulator, included the interaction of influences both on the metabolism of the and tumor. Most probably, the neurotransmitter modulation exerted an influence on cancerogenesis not only by regulation/disregulation of brain homeostasis, but also via a direct effect on intracellular processes during cell development and differentiation. Our results indicate that an increased monoamine level in the brain supports adaptive homeostatic mechanisms, which are among others responsible for the suppression of cancerogenesis.

Key words: experimental cancerogenesis, monoamines, caudate nuclei, piracetam.

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INTRODUCTION

Quite a number of retrospective and prospective studies of psychosomatic factors in cancer have been published (Bahnson et al., 1969, Le Shan, 1966). Among many major topics of the studies, research on the relationship between psychological factors and cancer has burgeoned and new lines of research have emerged (Reed and Jacopsen, 1988). Results from a number of investigations concerning psychological factors are being interpreted on two levels: in terms of their immediate relevance to clinical concerns and in terms of the support they offer for particular theoretical principles and/or constructs.

The experimental studies on a relationship between the CNS and cancerogenesis especially at the level of transmitters and cancerogenesis, stem from the important work of Alexandra von Metzler (1986). She showed that catecholamine agonists and cAMP agonists had specific antineoplastic effects. A combined tumor treatment with psychotonic neuropharmaceuticals and cytostatics was very successful, because psychotonics protected normal cells and acted as sensitizers on tumor cells. In the same work, she pointed out that changes of the electric activity of the CNS were evident during the intial phase of experimental tumor development as a consequence of altered transmitter metabolism. Interesting to note were data that the γ-amino-butyric acid content in the CNS (hypothalamus and hippocampus) increased, both in the initial phase of tumor growth, and afterwards, during development, and that the treatment of experimental animals with a GABA agonist accelerated tumor rate. Conversely, treatment of the animals with experimentally induced tumors with GABA antagonists and cAMP agonists reduced the tumor rate and retarded tumor growth (Metzler, 1972).

In a further development of the hypothesis about the role of monoamine factors in tumor genesis and subsequent consequences, we examined the effect of bilateral lesions of the caudate nuclei (as a important monoamine structure in the CNS), the effects of the psychotonic drug - Piracetam and the antineoplastic drug, cyclophosphamide, on survival time and incidence of metastases of tumor-bearing rats.

MATERIAL AND METHODS

We used 102 Wistar rats in the experiment during the last 4 years. The animals were injected with 1% 3-methylcholanthrene suspension in 10% Tylose, subcutaneously under the dorsal skin of the neck in doses of 3mg/animal. Within 6-9 months, after a single injection, the rats developed tumors, at the site of injection. The surgical removal was performed when tumors reached a size of 1-3 cm. After surgical extirpation of tumors different groups of animals were treated with bilateral lesions of the caudate nuclei, a psychoactive drug - Piracetam (administered by gastroesophageal tube 5 times/week, 100 mg/kg), cyclophos-

phamide (sc. single dose of 50mg/kg for female and 100mg/kg for male), or nothing (control - only surgical removal of tumors). Autopsy and histological examinations were done in all animals.

Bilateral lesions of the caudate nuclei (AP 0,5; ML 3,5; DV 5,5 - corresponding to the brain atlas of rats (Paxinos and Watsson, 1982) were produced by a 20-gauge stainless steel electrode, insulated except for 0,7 mm at the tip, through which 3,0 mA cathodal current was applied for 15 sec. from a DC constant current generator. Before lesions, the rats were anesthetized with pentobarbital (40 mg/kg) and placed in a stereotaxic apparatus (David Kopf). The lesioned animal received 5% dextrose in Ringer's solution as necessary to maintain nutrition during the first postoperative days. On completion of the experiments, lesioned animals were killed by an overdose of pentobarbital. Brains were perfused with 10% formaldehyde solution, cut in 20 μm coronal sections and stained with cresyl violet. Each lesioned site was localized with reference to the atlas of Paxinos and Watsson (1982).

RESULTS

Survival time (more than 120 days) was the greatest in group C (piracetam treatment after surgical removal of tumors), namely, 81,2% versus 12,5% from group B (bilateral lesions of the caudate nuclei after surgical removal of tumors), 50% of group A (only surgical removal of tumors) and 68,8% in group D (cyclophosphamide treatment after surgical removal of tumors) as shown in Table 1.

Table 1. Survival time > 120 days

Groups	n	Survival time > 120 days	
		Number	%
A	22	11	50.0
B	16	2	12.5
C	48	39	81.2
D	16	11	68.8

A - Only surgical removal of tumors - control group
B - Bilateral lesions of the caudate nuclei after surgical removal of tumors
C - Piracetam treatment after surgical removal of tumors
D - Cyclophosphamide treatment after surgical removal of tumors

The incidence of metastase in the animals which had a survival time more than 120 days, indicated that in group C there were no metastases (all the animals were without metastases), whereas in group B all of the animals had metastases. In group A 27,3% of the animals were without metastases and in group D 45,4% of the animals were without metastases as shown in Table 2.

Table 2. Incidence without metastasis (for animals which had a survival time >120 days)

Groups	n	Without metastasis	
		Number	%
A	11	3	27.3
B	2	0	0
C	39	39	100.0
D	11	5	45.4

A - Only surgical removal of tumors - control group
B - Bilateral lesions of the caudate nuclei after surgical removal of tumors
C - Piracetam treatment after surgical removal of tumors
D - Cyclophosphamide treatment after surgical removal of tumors

The statistic analysis is shown in Table 3.

Table 3. Fisher test

Groups	Groups				
	A	B	C	D	
A	########	0.015*	0.007*	0.139	SURVIVAL
B	0.577	########	<0.001*	0.002*	
C	<0.001*	0.001*	########	0.153	
D	0.238	0.359	<0.001*	########	
	INCIDENCE OF METASTASIS				

* - statisticaly significant

DISCUSSION

Cancer is a disease of multifactorial etiology and carcinogenesis is a multistep process. Thus the explanation of the high correlation of cancer and psychosocial as well neurological determinants includes at least three levels: brain homeostasis, neuroendocrine transduction, and target cell DNA response (Rakić et al., 1994). In a previous paper we pointed out that brain homeostasis is maintaind by different systems regulating behavior through the integration of basic neural processes of excitation and inhibition (Popov et al., 1994). Certain experimental and clinical data indicate that stress is involved in the genesis of behavioral and psychosomatic impairment, via its disruptive effects on brain monoamine neurotransmitters (Glavin, 1985). In our experiment, in group C (piracetam treatment after surgical removal of tumors), survival time and incidence of metastasis was the most favourable compared with group B (bilateral lesions of the caudate nuclei after surgical removal of tumors), group D (cyclophosphamide treatment after surgical removal of tumors) or group A (control group). The differences between group C and group B are highly statistically significant. It is evident that surgical removal of tumors, accompanied by treatment with a pshychoactive drug or a cytostatic, had a favorable effect by prolonging survival time. Piracetam does not affect cell proliferation and no

primary or secondary resistance developed during treatment, contrary to the case with most chemotherapeutics. Thus, its effectiveness described here, including the absence of metastases, has a special significance.

The experimental data presented indicate that tumor growth may be inhibited by influencing the transmitter content in the CNS (Metzler, 1986). Most probably, the neurotransmitter modulation exerted an influence on cancerogenesis not only through regulation/disregulation of brain homeostasis, but also via direct effects on intracellular processes during cell development and differentiation. The transfer of information from a tumor at the periphery to the CNS and conversely, from the CNS to a tumor may use a humoral or neuronal route to neuronal cells leading to effecting central disorders. Our results indicate that drugs aftecting the CNS could produce a reverse transfer from neuronal to non-neuronal cells leading to a decreased tumor rate or occasionally remission (Popov et al., 1995).

The altered brain homeostasis caused by emotional stress as well as in other pathological conditions, followed by a decreased level of catecholamines, produces remarkable changes in neuroendocrine transduction, increased stimulation of ACTH and gonadotropins, followed by steroidogenesis and elevation of both corticoids and sex steroids. All those functional and structural changes mediated by steroids after the regulation of gene expression and the target cell DNA with multiple and multistep changes (initiations of mutations, insertion of viral oncogenes, viral promoters, activation of C-oncogenes, to the activation of dormant cancer cells, tumorigenesis, progression and metastasis i Grossarth Maticek et al., 1991). The integrity of the system, brain homeostasis-neuroendocrine transduction-target cell DNA, plays an active part in the general adaptation and survival of the organism. The continuous regulatory deficiency in adaptive homeostasis triggers unvearable functional and metabolic, including immunological, disturbances, important for promotion of cancerogenesis, progression of tumor growth and metastasis. Consequently, an increased level of catecholamines operates in the reverse direction in adaptive homeostasis, triggering the systemic mechanisms responsible for the suppression of cancerogenesis.

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EFEKAT BILATERALNIH LEZIJA NUKLEUSA CAUDATUSA I PSIHOSTIMULATORNI EFEKAT PIRACETAMA NA RAZVOJ KANCERA IZAZVANOG METILHOLANTRENOM KOD PACOVA

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SADRŽAJ

Dosta je teorijskih rasprava o uticaju monoaminskih neurotransmitera CNSa na kancerogenezu. Naše istraživanje je imalo cilj da uporedi efekat bilateralnih lezija Nukleusa caudatusa (kao značajne monoaminske strukture u CNS-u), psihotoničnog efekta piracetama i davanja citostatika ciklofosfamida u odnosu na preživljavanje i metastaziranje u eksperimentalnoj kancerogenezi kod metilholantrenom indukovanih tumora pacova. Sto dva Wistar pacova je upotrebljeno. Posle tumorske indukcije 3-metilholantrenom, rađena je hirurška ekstirpacija primarnog tumora, da bi se zatim životinje delile u različite grupe u kojima su rađene bilateralne lezije nukleusa Caudatusa, priman piracetam, ciklofosfamid ili su predstavljale kontrolnu grupu (nisu primale ništa). Sve životinje su na kraju bile obdukovane i histopatološki je verifikovana metastatska diseminacija. Najduže preživljavanje je postignuto u piracetamskoj grupi gde duže od 120 dana preživljava 81,2% životinja, 12,5% u grupi u kojoj su rađene lezije nukleusa Caudatusa, 68,8% u ciklofosfamidskoj i 50% u kontrolnoj grupi. U piracetamskoj grupi sve životinja koje su preživele 120 dana su bile bez metastaza u odnosu na grupu životinja sa lezijama Caudatusa u kojoj nisu registrovane životinje bez metastaza. U ciklofosfamidskoj je bilo 45,4% i 27,3% u kontrolnoj grupi životinja koje su preživljavale duže od 120 dana a bile su bez metastaza.

Razlika u preživljavanju i metastaziranju u korist monoaminskih stimulatora može se tumačiti antitumorskim efektom koji monoamini ostvaruju preko promena u CNSu kao i promenama u metabolizmu tumora, ostvarujući svoj uticaj ne samo na procese regulacije/deregulacije moždane homeostaze već i direktno na intraćeliske procese u toku ćelijskog razvoja i diferencijacije.

Naši rezultati ukazuju na mogućnost da povišenje nivoa monoamina u CNSu, preko podrške očuvanju homeostatskih mehanizama, može uticati na zaustavljanje razvoja kao i metastaziranja tumora.