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Short communication

Acute treatment with doxorubicin induced neurochemical impairment of the function of dopamine system in rat brain structures

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ABSTRACT

Background: The clinical studies have shown that chemotherapy may impair cognitive functions especially in the patients treated for breast cancer. It should be mention that only few studies have made use of animals to investigate the effects of chemotherapy on the brain function. Doxorubicin (Adriamycin) is an anthracycline antibiotic commonly used for chemotherapy of breast cancer.
Methods: This study examined the effect of doxorubicin (1.5 and 3.0 mg/kg *ip*) after acute administration on the levels of dopamine, noradrenaline, serotonin and their metabolites in the rat brain structures connected with cognition and psychiatric disorders.
Results: The data indicate that doxorubicin produced a significant and specific for the dopamine system inhibition of its activity in the investigated structures connected with the fall of dopamine concentration (decrease from 25 to 30% in the frontal cortex; from 30 to 60% in the hippocampus and about 20% of the control in the striatum, $p < 0.05$) and its extraneuronal metabolite, 3-MT (from 35% in the frontal cortex to 60% in the hippocampus of the control level, $p < 0.01$). However, doxorubicin did not affect others monoaminergic transmitters in the brain: noradrenaline and serotonin.
Conclusion: Summing up, these data indicate that a single injection of doxorubicin produced a clear and significant inhibition of dopamine system activity in all investigated structures with the strongest effect in the hippocampus what may lead to the disturbances of the cognitive functions at the patients treated for cancer. Moreover, such treatment did not significantly affect others monoaminergic transmitters such as noradrenaline and serotonin.

Introduction

An increasing number of clinical studies have shown that chemotherapy may impair cognitive function after adjuvant chemotherapy for non-CNS tumors. Impairment of cognitive performance can already be noticed during treatment and may persist many years after completion [1,2]. "Chemo brain" or "chemo fog" refers to deterioration of cognitive functions of patients after cessation of chemotherapy. Clinical studies have shown impairments of memory, executive functions, attention and processing speed noted especially in the patients treated for breast cancer [1,3].

Doxorubicin (Adriamycin) is an anthracycline antibiotic commonly used for chemotherapy of breast cancer and during this treatment patients reveal decrease of overall cognitive scores [4], and decrements in attention, visuospatial skills, and delayed memory [2]. Administration of doxorubicin to rodents is associated with hippocampal-related learning and memory impairments [5]. Consistently, the recent study shows that doxorubicin impairs hippocampal-based memory function on the novel place recognition and contextual fear conditioning task in rats, as well as it disrupts hippocampal neurogenesis [6].

In an attempt to evaluate some of these factors we examined the effect of doxorubicin on the monoaminergic system in the rat brain. We investigated the effect of doxorubicin on the levels of dopamine, noradrenaline, serotonin and their metabolites in the brain structures connected with cognition and psychiatric disorders i.e. frontal cortex, hippocampus, striatum.

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Materials and methods

Animals

The experiments were carried out on male Wistar rats (Charles River) of initial body weight 230-240 g (about 7 weeks old). The animals were kept under standard laboratory conditions with free access to laboratory food and tap water, at room temperature of 22 °C with an artificial day–night cycle (12/12 h, light on at 7 a.m.).

All the procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were granted an approval from the Bioethics Commission as compliant with Polish Law.

Drugs

Doxorubicin hydrochloride (Adriblastina; Pfizer, Mediolan, Italy). Doxorubicin (1.5 mg/kg and 3 mg/kg) was administered intraperitoneally (*ip*). The control group received 0.9% saline *ip*.

Neurochemical ex vivo *studies*

The neurochemical analysis was carried out 3 h after acute administration of doxorubicin in the both doses (1.5 and 3 mg/kg *ip*) as well as 24 h after a higher dose. The rats were killed by decapitation and the brain was rapidly removed and dissected on different brain structures (frontal cortex, hippocamp, striatum) on an ice-cold glass plate. The structures were frozen on solid CO_2 (−70 °C) until used for biochemical assays. Dopamine (DA) and its metabolites, the intraneuronal, 3,4-dihydroxyphenylacetic acid (DOPAC); the extraneuronal, 3-methoxytyramine (3-MT) and the final metabolite, homovanillic acid (HVA); noradrenaline (NA) and its main extraneuronal brain metabolite, normetanephrine (NM) and serotonin (5-HT) and its intraneuronal metabolite 5-hydroxyindolacetic acid (5-HIAA) were assayed by means of high-performance liquid chromatography (HPLC) with electrochemical detection (ED). The tissue samples were weighted and homogenized in ice-cold 0.1 M trichloroacetic acid containing 0.05 mM ascorbic acid. After centrifugation (10,000 × *g*, 5 min), the supernatants were filtered through RC58 0.2 μm cellulose membranes (Bioanalytical Systems, West Lafayette, IN, USA). The chromatograph HP 1050 (Hewlett-Packard, Golden, CO, USA) was equipped with Hypersil columns BDS-C18 (4 mm × 100 mm, 3 μm). The mobile phase consisted of 0.05 M citrate-phosphate buffer, pH 3.5; 0.1 mM EDTA; 1 mM sodium octyl sulfonate and 3.5% methanol. The flow rate was maintained at 1 ml/min. DA, NA and 5-HT and their metabolites were quantified by peak area comparisons with standards run on the day of analysis (ChemStation, Hewlett-Packard software computer program).

Statistical analysis

The results were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's *post-hoc* test when appropriate. The data were considered statistically significant when $p < 0.05$. The rate of dopamine COMT-dependent O-methylation and as a factor of dopamine release the ratio: [3-MT]/[dopamine] × 100. Analogously, the rate of noradrenaline metabolism was expressed as the ratio: [NM]/[noradrenaline] × 100 and serotonin as the ratio: [5-HIAA]/[serotonin] × 100. The indices were calculated using concentrations from individual tissue samples (n = 5-6) [7].

Results

The effect of doxorubicin on the concentration of DA and its metabolites, and on the rate of DA catabolism in rat brain structures

The one-way ANOVA showed a significant effect of doxorubicin administration on DA and its extraneuronal metabolite (3-MT) concentrations in the brain structures (Table 1). The Duncan *post-hoc* test indicated that doxorubicin (1.5 and 3.0 mg/kg) significantly decreased (from 30 to 50%; $p < 0.05$) DA and its extraneuronal metabolite, 3-MT (from 30 to 60%; $p < 0.05$) concentrations in the brain structures: frontal cortex, hippocamp and striatum. The effect of doxorubicin was not dose related, and in

Table 1
The effect of doxorubicin on the dopamine metabolism in the rat brain structures.

Treatment (mg/kg)	DA (ng/g t)	DOPAC (ng/g t)	3-MT (ng/g t)	HVA (ng/g t)	[3-MT]/[DA]
Frontal cortex					
Saline	992 ± 76	285 ± 23	50 ± 8	141 ± 12	5.0 ± 0.88
Doxorubicin 1.5 (3 h)	710 ± 35*	204 ± 14	29 ± 8*	129 ± 17	4.0 ± 1.80
Doxorubicin 3.0 (3 h)	741 ± 54*	228 ± 38	33 ± 3*	118 ± 16	5.1 ± 0.41
Doxorubicin 3.0 (24 h)	780 ± 96	221 ± 25	34 ± 7*	122 ± 10	4.0 ± 0.75
F	$F_{(3/17)} = 3.801$	$F_{(3/17)} = 1.560$	$F_{(3/17)} = 4.581$	$F_{(3/17)} = 0.583$	$F_{(3/17)} = 0.372$
	$p < 0.0567$	*N.S.*	$p < 0.0503$	*N.S.*	*N.S.*
Hippocampus					
Saline	51 ± 5	12 ± 1.5	5.0 ± 1.3	11.0 ± 1.7	10.0 ± 2.2
Doxorubicin 1.5 (3 h)	21 ± 5**	8 ± 1.1	2.0 ± 0.4**	7.5 ± 1.5	10.2 ± 2.6
Doxorubicin 3.0 (3 h)	28 ± 4**	8 ± 1.6	1.9 ± 0.3**	7.8 ± 1.4	8.0 ± 2.7
Doxorubicin 3.0 (24 h)	35 ± 4*	9 ± 1.0	2.0 ± 0.3**	8.5 ± 1.0	5.4 ± 1.0
F	$F_{(3/16)} = 6.831$	$F_{(3/16)} = 2.336$	$F_{(3/16)} = 4.589$	$F_{(3/16)} = 2.405$	$F_{(3/16)} = 1.101$
	$p < 0.0047$	*N.S.*	$p < 0.0165$	*N.S.*	*N.S.*
Striatum					
Saline	11,027 ± 312	1481 ± 33	452 ± 44	829 ± 61	4.0 ± 0.31
Doxorubicin 1.5 (3 h)	9195 ± 262*	1355 ± 65	353 ± 40	715 ± 98	4.0 ± 0.52
Doxorubicin 3.0 (3 h)	8706 ± 395**	1263 ± 40	384 ± 31	679 ± 44	3.8 ± 0.13
Doxorubicin 3.0 (24 h)	10,502 ± 502	1651 ± 85	315 ± 19	825 ± 33	2.9 ± 0.11*
F	$F_{(3/16)} = 5.899$	$F_{(3/16)} = 2.899$	$F_{(3/16)} = 2.926$	$F_{(3/16)} = 1.374$	$F_{(3/16)} = 3.956$
	$p < 0.0081$	*N.S.*	*N.S.*	*N.S.*	$p < 0.032$

Doxorubicin (1.5 mg/kg and 3 mg/kg) was administered once (acute treatment) and animals were decapitated 3 h and 24 h after injection. Control group (Saline) received 0.9% saline. The concentration (ng/g wet tissue) of dopamine and its metabolites was measured in the rat frontal cortex, hippocampus and striatum. The data are the means ± SEM (n = 5–6). The ratio: [3-MT]/[dopamine] × 100 demonstrated the rate of dopamine COMT-dependent O-methylation as a factor of dopamine release. The results were analyzed by means of one-way ANOVA analysis of variance, followed when appropriate, by *post-hoc* Duncan's test.

* Statistical significance: $p < 0.05$.

** Statistical significance: $p < 0.01$ *vs.* saline group.