CHRONOBIOLOGICAL CHANGES INDUCED BY THE GALLIUM COMPLEX C(24) IN SOME HEMATOLOGICAL PARAMETERS IN RATS

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ABSTRACT

In the present research the authors proposed to monitor possible chronobiological changes in some hematological parameters (erythrocytes, leukocytes and thrombocytes) of Wistar strain rats induced by the novel gallium complex - C(24). For this purpose a morning (m) and an evening (e) animal series were designed, each including a control (C_p) and an experimental (E) group. Animals of the control groups (C_p-m, C_p-e) were injected intraperitoneally with polyethylene glycol and those from the experimental groups (E-m, E-e) with the novel gallium complex in the morning and in the evening. At 48 hours after the administration of substances the animals were anesthetized and blood samples were collected for biochemical and hematological analysis.

Erythrocytes, leukocytes and thrombocytes parameters were determined. The resulted values showed an increase in most of erythrocytes parameters in E-m group and a slight decrease of all parameters in E-e group. As to leukocytes parameters an increase of most values both in E-m and E-e groups was recorded. Platelets parameters indicated decrease both in E-m and E-e groups, being more evident in case of platelets number.

Key words : gallium complex C(24), chronobiology, hematological parameters

INTRODUCTION

In chronobiology and chronobiochemistry one can distinguish three types of rhythms : a) ultradian - from some seconds to some hours; b) circadian - about 24 hours; c) infradian - from some days to months or year (Hayes et al., 1990; Schwob, 2007).

In the last period more and more experimental and clinical studies are dealing with the anticancer effects of various gallium salts and complexes (Haiduc and Silvestru, 1990; Chitambar, 2010). This trend is due to the fact that gallium is the second metallic element after platinum with evident anticancer effects (Bernstein, 2005).

The aim of the present paper was to evidence possible changes in some hematological parameters after a period of 48 hrs (including two circadian cycles) from the administration of

the gallium complex C(24) with the molecular weight 820 Da - synthetized at the Department of Inorganic Chemistry of the University "Babeş-Bolyai" Cluj-Napoca (Vălean et al., 2009).

MATERIALS AND METHODS

Experimental design. Wistar strain rats, divided into a morning series (administration time 7 a.m.) and an evening series (administration time 7 p.m.) were used. Afterwards each series were divided in two control groups, i.e. one morning (C_p-m) and one evening (C_p-e) - the animals receiving intraperitoneally (i.p.) a solution with Et-OH 40% : PEG 400 in the ratio of 1 : 1.5 (Gârban et al., 2012). It is to mention that polyethylene glycol (PEG) is suitable for solubilizing of various compounds of pharmaceutical and nutritional interest as well as xenobiotics (Milton, 1992; Smolinske, 1992; Gârban, 2007). Also, there were constituted the morning (E-m) and evening (E-e) experimental groups. Animals (E-m and E-e) were injected i.p. with 0.25 mg/mL of the compound C(24) in solution with Et-OH / PEG. A quantity of 1 mL / 100 g body weight (b.wt.), i.e. 2.5 mg/kg b.wt. was injected. Each animal group consisted of 3 males and 3 females. The administered compund noted C(24) was an *amonium gallium complex of phosphinobisthiolato P,S,S pincer ligand* with the formula :

[NEt3H][Ga{PPh(2-SC6H4)2-k3S,S',P}-{PPh(2-SC6H4)2-k2S,S'}]

The experiment lasted 48 hrs starting from the administered substances. At the end of the experiment blood samples were collected for analysis and the animals were euthanized by an Anesteran (purchaed from Rompharm Co., Bucharest-Romania) overdose. Requirements for the protection of animals used in scientific or other experiments were respected according to Council Directive 86/609/EEC of 24 November 1986 and National Governmental Ordinance No.37/30.01.2002.

Hematological investigations: Using an automated „Abacus Junior Vet" type analyzer where a quantity of 25 µl whole blood was introduced we obtained data on parameters regarding: red blood cells (erythrocytes); white blood cells (leukocytes) and platelets (thrombocytes). Parameters of red blood cells were: total number-RBC; hemoglobin-HGB; hematocrit-HTC; red blood cell indices-MCV; mean corpuscular hemoglobin-MCH; mean corpuscular hemoglobin concentration-MCHC; red cell distribution width-RDWc. Parameters of white blood cells were: WBC-total number; LYM-lymphocytes; MID-medium size cells count; GRA-granulocytes; LY%-lymphocytes percentage; MI%-medium size cells percentage; GR%-granulocytes percentage. Parameters for platelets were: PLT-number of platelets; PCT-platelet percentage; MPV-mean platelets volume; PDWc-platelet distribution width.

Statistical evaluation. Mean values (X) and standard deviations (SD) of all the experimental data were determined. Data were evaluated using a conventional statistical procedure, the analysis of variance (ANOVA).

RESULTS AND DISCUSSIONS

Parameters of red blood cells are the main indicators of the iron status of the organism and can be modified either by the time-period of collection (Gârban et al., 1989; Pocock et al., 1989; Sanni et al., 2000) or by various factors such as: acute hemorrhage, lack of substances needed for RBC production, action of various xenobiotics a.o. The oxyphoretic capacity of RBC is important in order to prevent tissue hypoxia and the consequent disturbances. Our results on the effects of the gallium complex C(24) on erythrocyte parameters are presented in table 1 and table 2.

Table 1. Erythrocyte parameters obtained in the morning animal series

Groups	n	RBC ($\bullet 10^{12}$/L) X ± SD	HGB (g/dL) X ± SD	HCT (%) X ± SD	MCV (fl) X ± SD	MCH% (pg) X ± SD	MCHC (g/dL) X ± SD	RDW_C (%) X ± SD
C_p - m	6	7.83 ± 1.87	12.58 ± 3.32	38.24 ± 5.43	49.50 ± 2.25	15.98 ± 0.53	32.41 ± 1.63	17.11 ± 0.65
E - m	6	8.80 ± 0.88	14.01 ± 1.36	40.18 ± 2.24	47.50 ± 2.07	15.95 ± 0.51	33.53 ± 1.13	18.23 ± 1.49
ΔX		+ 0.97	+ 1.43	+ 1.94	- 2.00	- 0.03	+ 1.12	+ 1.12

Table 2. Erythrocyte parameters obtained in the evening animal series

Groups	n	RBC ($\bullet 10^{12}$/L) X ± SD	HGB (g/dL) X ± SD	HCT (%) X ± SD	MCV (fl) X ± SD	MCH% (pg) X ± SD	MCHC (g/dL) X ± SD	RDW_C (%) X ± SD
C_p - e	6	9.17 ± 0.45	14.90 ± 1.05	44.13 ± 1.55	48.16 ± 2.04	16.23 ± 0.77	33.75 ± 0.78	18.63 ± 1.59
E - e	6	8.58 ± 0.76	13.78 ± 0.92	41.12 ± 1.51	48.00 ± 1.41	16.08 ± 0.74	33.50 ± 0.91	17.52 ± 0.53
ΔX		- 0.59	- 1.12	- 3.01	- 0.16	- 0.15	- 0.25	- 1.11

In vivo studies using Ga compounds revealed that Ga is present mostly in blood serum and in small quantites in leukocytes (Bernstein, 2005). Parameters of white blood cells have an important role in the defending processes of the organism, e.g. phagocytosis. In table 4 and table 4 are given the obtained results regarding these parameters after C(24) injection.

Table 3. Leukocytic parameters obtained in the morning animal series

Groups	n	WBC ($\bullet 10^9$ / L) X ± SD	LYM ($\bullet 10^9$ / L) X ± SD	MID ($\bullet 10^9$ / L) X ± SD	GRA ($\bullet 10^9$ / L) X ± SD	LY (%) X ± SD	MI (%) X ± SD	GR (%) X ± SD
C_p - m	6	10.70 ± 3.12	8.15 ± 2.15	0.32 ± 0.18	2.21 ± 1.02	76.83 ± 3.05	3.23 ± 2.91	19.93 ± 3.11
E - m	6	13.06 ± 3.82	10.57 ± 3.19	0.33 ± 0.21	2.15 ± 0.71	81.05 ± 3.05	3.61 ± 2.43	17.73 ± 3.15
ΔX		+ 2.36	+ 2.42	+ 0.01	- 0.06	+ 4.22	+ 0.38	- 2.20

Table 4. Leukocytic parameters obtained in the evening animal series

Groups	n	WBC ($\bullet 10^9$ / L) X ± SD	LYM ($\bullet 10^9$ / L) X ± SD	MID ($\bullet 10^9$ / L) X ± SD	GRA ($\bullet 10^9$ / L) X ± SD	LY (%) X ± SD	MI (%) X ± SD	GR (%) X ± SD
C_p - e	6	7.66 ± 2.76	5.51 ± 2.52	0.34 ± 0.18	1.80 ± 0.89	70.58 ± 8.09	4.67 ± 2.98	24.75 ± 7.72
E - e	6	9.58 ± 4.01	6.80 ± 2.89	0.42 ± 0.26	2.35 ± 1.37	71.85 ± 5.77	4.22 ± 2.09	23.93 ± 5.23
ΔX		+ 1.92	+ 1.29	+ 0.08	+ 0.55	+ 1.27	- 0.45	- 0.82

Platelets or thrombocytes are small cell fragments with a short life time which circulate in the blood and are involved in hemostasis. The results obtained in this experiment are depicted in table 5 and 6.

Table 5. Parameters of platelets obtained in the morning animal series

Groups	n	PLT ($\bullet 10^9$) X ± SD	PCT (%) X ± SD	MPV (fL) X ± SD	PDW_C (%) X ± SD
C_s - m	6	503.00 ± 379.00	0.29 ± 0.20	6.03 ± 0.80	30.60 ± 1.45
E - m	6	477.00 ± 245.00	0.27 ± 0.13	5.91 ± 0.79	30.51 ± 1.89
Δ X		- 26.00	- 0.02	- 0.12	- 0.09

Table 6. Parameters of platelets obtained in the evening animal series

Groups	n	PLT ($\bullet 10^9$) X ± SD	PCT (%) X ± SD	MPV (fL) X ± SD	PDW_C (%) X ± SD
C_s - e	6	595.80 ± 133.00	0.34 ± 0.08	5.56 ± 0.16	28.78 ± 0.38
E - e	6	542.20 ± 140.80	0.30 ± 0.07	5.53 ± 0.28	28.77 ± 0.61
Δ X		- 53.60	- 0.04	- 0.03	- 0.01

Both in the morning (E-m) and in the evening (E-e) experimental groups the number of thrombocytes was decreased when compared with the control groups. The decrease was more evident in case of the evening experimental group.

CONCLUSIONS

1. The administered gallium complex C(24) on the parameters of red blood cells (RBC) showed the followings: statistically not significant increase of RBC, HGB, HCT, MCHC and RDW_C and decrease of MC and MCT in the morning series. In the evening series all the parameters were slowly decreased.
2. Regarding the effects of gallium complex C(24) on the parameters of white blood cells (WBC) an increase of values both in the morning and evening series were found with some exceptions.Thus, GRA decreased in the morning, MI decreased in the evening while GR decreased both in the morning and evening series.
3. Parameters concerning the platelets (PLT) under the action of the gallium complex C(24) revealed decrease in both animal series. More important decrease was found in case of the platelets number in the evening experimental series.

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