ASPECTS OF THE HOMEOSTASIS CHANGES INDUCED BY THE GALLIUM COMPLEX C(24) IN EXPERIMENTS ON RATS

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Abstract
The action of the Ga complex C(24) on some serum biochemical parameters in rats was studied in two different day times, i.e. morning and evening. There were analyzed : total serum proteins (PRO); serum albumin (ALB); serum non-protein nitrogenous compounds, i.e. uric acid (UA), creatinine (CRE), blood ureea nitrogen (BUN); calcemia and magnesiemia. The obtained values and calculation of differences made possible to evidence homeostasis changes occurring in two direction: conditioned by time (choronobiochemistry) and conditioned by the administered xenobiotic (i.e. the studied Ga complex).

Keywords:homeostasis, serum biochemical parameters, gallium complex C(24)

Introduction
Investigations regarding blood biochemical parameters are of special interest to metabolomics and metallomics because blood is the main tissue that transports the metabolites and xenobiotics (including the pharmaceutical ones, too) in the organism.
Such studies can allow to evidence and monitor homeostasis changes caused by various chemical xenobiotics of nutritional (e.g. food contaminants) and pharmaceutical interest (chemotherapeutics) and can offer useful information in elucidating their structures, properties, mechanism of actionat molecular and tissue level (Gielen and Tienkink, 2005).
All the above mentioned investigations are of importance in nutrition, toxicology, pharmacology and, evidently in biochemistry and xenobiochemistry (Testa, 1995). In the last two decades there were performed numerous investigations on Ga compounds in order to use them in therapy, implicitly in certain form of cancer (Haiduc and Silvestru, 1989; Collery et al., 2002).
Certain studies had in view distinctly the following inorganic compounds: gallium nitrate, chloride and sulfate (Bernstein, 1998; Chitambar, 2012). Also, there were investigated the effects induced by some organometallic compounds as: gallium maltolate,gallium 8-quinolinolate (Collery et al., 2002), gallium protoporphirin IX (Arivett et al., 2015).

The aim of this study was to evidence the effects induced in rats by the new gallium complex C(24) considered as a xenobiotic.

Experimental

Chemicals.

Experiments were performed on the actions of the organometallic gallium complex with the formula: [NEt3H][Ga{PPh(2-SC6H4)2-k3S,S',P}-{PPh(2-SC6H4)2-k2S,S'}]

This complex is noted usually C(24) and has a molecular weight of 820.71 Da - details see Vălean et al., 2009. The gallium complex C(24) was solved in a mixture of co-solvents consisting of: water-ethanol-polyethylen glycol (PEG) 400. In biomedical experiments and in some pharmaceuticals the usage of PEG is a regular practice (Milton, 1992; Gârban et al., 2013 a). In this study there were used ethanol-C_2H_5-OH with the molecular weight46.07producedbyS.C."P.A.M. Corporation" S.R.L., Romaniaandpolyethyleneglycol HO $(C_2H_4)_nH$ with the molecular weight380-420and density of1.13g/cm^3, produced bySCharlabSL., Pol. Ind. Mas d`enCisa, Sentmenat, Barcelona, Spain.

Experimental design.

In this study Wistar strain albino rats weighing 100-120 g were used. They were fed with commercial dry pellets manufactured by S.C. Freman Oradea and received tap water *ad libitum*. Two series of animals were composed: a morning (m) one - administration of substance at 7 a.m. and an evening (e) one - administration of substance at 7 p.m. Each series consisted of two groups (each with 6 animals): control (C) and experimental (E) . Animals of morning control group C_m and evening control group C_e were injected intraperitoneally (i.p.) with physiological saline 1 mL / 100 g b.w. Animals of morning experimental group E_m and evening experimental group E_e were injected i.p. with a solution containing the Ga complex C(24) dissolved in the core-solution a „co-solvent" containing Et-OH 40% : PEG 400 at the ratio 1: 1.5 (practically the solvent for the Ga complex, i.e. its carrier).The concentration of the administered gallium complex C(24) in solution was 0.25 mg/mL, each animal receiving 1 mL / 100 g b.w. (i.e. 2.5 mg/kg b.w.). During the injection of the substances the animals were anesthetized by inhalatory administration of Anesteran (Rompharm Co. Bucharest). At the end of the experiment, i.e. at 48 hrs the animals were anesthetized again and blood samples were collected for biochemical and hematological analysis. Determinations were carried out in identical conditions in the morning / evening. The investigations were in accordance with the ethical standards laid down in the Council Directive 86/609/EECregarding the protection of animals used for experimental and other scientific purposes.

Investigation of the biochemical parameters.

The biochemical parameters were determined by spectrophotometric methods used in clinical chemistry. For this purpose the biochemical "Spotchem" Analyzer was used with specific reagent strips manufactured by „Arkray Factory Inc." (Koji-Japan). Thus, the followings were considered: total serum proteins (PRO); albumin (ALB); non-protein nitrogen compounds, i.e. uric acid (UA), creatinine (CRT), blood urea nitrogen (BUN); calcemia and magnesiemia. Details on the analytical methods were presented in a previous paper (Gârban et al., 2013 b).

Statistical evaluation.

Analytical data were processed by determining the mean values (X) and standard deviations (SD). To analyse the differences between group means the analysis of variance (ANOVA) test was applied by using a SSPS IBM Statistic 19.0 software package.

Results and discussions

The experiments presented in this paper pursued to highlight possible disturbances in the homeostasis of the mentioned serum biochemical parameters induced by the new gallium complex C(24).

It is known that certain gallium compounds are used not only in the treatment of tumors but also in some infective diseases (Antunes et al., 2012). Usage of Ga(III) as compound with anti-infective action is considered as a "Trojan Horse" strategy in the interactions occurring in the living tissue (Arivett et al., 2015) .

One of issues was to assess the "effects conditioned by time"- with importance in chronobiochemistry- both in case of control groups C_m and C_e and of experimental groups E_m and E_eThese effects were denotedas (ΔX_1) and represent the differences between the means of the morning and evening parameters. A second issue was the evaluation of the "effects conditioned by the substance" (the xenobiotic with gallium) and denoted as (ΔX_2) being the differences between the means of control groups and experimental groups.

Results of investigations concerning serum proteins and albumin in rats injected with the gallium complex C(24) are given in Table 1.

Normal values of proteinemia and albuminemia in rats, according to Car et al. (2006) are 5.5-6.6 g/dL respectively 4.0-4.8 g/dL. Other literature data give different values, e.g. 5.6-7.6 g/dL for total serum proteins and 3.8-4.8 g/dL for serum albumin (http://www.ratfanclub.org./values.html).

Our data for PRO and ALB showed a mild evening increase in case of control groups and an evening decrease in the experimental groups (ΔX_1). The obtained values were statistically non-significant. These findings could be attributed to the chronobiological specificity of protein synthesis in animals (Gârban, 2015). Such data were mentioned in humans, too (Haus et al. 1993).

Table 1 Homeostasis changes in serum protein and albumin

Serum parameters	UM	Groups (n=6)	X ± SD	Groups (n=6)	X ± SD	ΔX_1
PRO	g/dL	C_m	5.72 ± 0.30	C_e	5.90 ± 0.34	+ 0.18
		E_m	5.67 ± 0.24	E_e	5.65 ± 0.33	- 0.02
		ΔX_2	- 0.05	–	- 0.25	–
ALB	g/dL	C_m	3.18 ± 0.32	C_e	3.26 ± 0.08	+ 0.08
		E_m	3.08 ± 0.13	E_e	2.88 ± 0.27	- 0.20
		ΔX_2	- 0.10	–	- 0.38	–

Analyzing the obtained data of PRO and ALB in the experimental groups versus to those of control groups one can observe that the gallium complex C(24) induceda light decrease (see ΔX_2) both in the morning and evening series.

Concerning the results of serum non-protein nitrogen metabolites, i.e. uric acid (UA), creatinine (CRE) and blood urea nitrogen (BUN) as well as those of calcium and magnesium are presented in Table 2.

Table 2 Homeostasis changes in the serum non-protein nitrogen metabolites, calcium and magnesium

Serum parameters	UM	Groups (n=6)	X ± SD	Groups (n=6)	X ± SD	ΔX_1
UA	mg/dL	C_m	0.64 ± 0.09	C_e	0.62 ± 0.08	- 0.02
		E_m	0.70 ± 0.12	E_e	0.64 ± 0.09	- 0.06
		ΔX_2	+ 0.06	–	+ 0.02	–
CRE	mg/dL	C_m	0.58 ± 0.08	C_e	0.60 ± 0.15	+ 0.02
		E_m	0.62 ± 0.10	E_e	0.63 ± 0.15	+ 0.01
		ΔX_2	+ 0.04	–	+ 0.03	–
BUN	mg/dL	C_m	15.66 ± 3.26	C_e	16.83 ± 3.43	+ 1.17
		E_m	19.83 ± 4.16	E_e	17.66 ± 3.72	- 2.17
		ΔX_2	+ 4.17	–	+ 0.83	–
Calcium	mg/dL	C_m	11.48 ± 0.31	C_e	11.23 ± 0.19	- 0.25
		E_m	11.46 ± 0.38	E_e	11.68 ± 0.42	+ 0.22
		ΔX_2	- 0.02	–	+ 0.45	–
Magnesium	mg/dL	C_m	1.96 ± 0.22	C_e	1.95 ± 0.12	- 0.01
		E_m	1.82 ± 1.54	E_e	1.96 ± 0.05	+ 0.14
		ΔX_2	- 0.14	–	+ 0.01	–

The results of the experiment showed chronobiochemical changes in the control groups by the increase of the evening values of CRE and BUN as well as the decrease of UA. In case of experimental groups UA and BUN decreased while CRE increased in the evening series. As to the values of Ca and Mg an evening decrease in the control groups and increase in the experimental groups were found (see ΔX_1). Chronobiological changes by Ga involvement in substitution of Fe, Ca, Mg, etc. were reported by Bernstein (1998).

When evaluating the effects induced by the gallium complex C(24) - evidenced by the differences ΔX_2 - one can remark increases of UA, CRE, BUN in all experimental groups. Values of serum Ca and Mg showed a decrease in the morning and increase in the evening. Experiments on rats by i.p. injection of Ga salts (nitrate) made by Chitambar (2012), showed a decrease in serum Ca and inhibition of bone resorbtion.

This evaluation of homeostasis changes explains, per se, the interest toward the effects conditioned by time and induced by various chemical compounds (xenobiotics) in the animal organism which can be extrapolated to humans, too.

Conclusions

1. Data conditioned by time (chronobiochemical) highlighted in control groups C_m and C_e a mild increase of PRO, ALB, CRE and BUN while in case of experimental groups E_m and E_e only CRE, Ca and Mg increased. These data have predictive character for the study of the chronopharmacological applications.
2. The results conditioned by the xenobiotic (i.e. the gallium complex) showed decrease of PRO and ALB while UA, CRE and BUN increased. These data point out the action of Ga C(24) on the renal function. In case of Ca and Mg a morning decrease and evening increase were found.

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