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Polyethylene Glycol Used in a Study Regarding the In Vivo Effects of the Organometallic Gallium Complex C(85)

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Abstract: The paper presents the use of polyethylene glycol (PEG) in a solvent mixture for solubilizing the organometallic gallium complex C (85) in order to investigate its effects on an animal experimental model. Changes of biochemical homeostasis of certain blood parameters revealed the effects induced by the intraperitoneally administered Ga complex in Wistar strain rats in two different period of day. In this regard there were detected changes in serum protein and albumin as well as in serum calcium and magnesium. Variations were noticed also in the concentration of non-protein nitrogenous substances (uric acid, creatinine and blood urea nitrogen).

Keywords: PEG - gallium complex, in vivo experiment

1. Introduction

Technical and medical applications of polyethylene glycol (PEG) had retained the attention due to the structural features and physico-chemical properties of this macromolecule [1, 2, 3]. In the last two decades PEG gained an extensive use in the field of foodstuffs and pharmaceuticals **[**4, 5]

Polyethylene glycol along with glycol, alcohol and water is suitable for solubilizing of various compounds of pharmaceutical and nutritional interest. Solubilization is usually performed in a mixture of solvents. It was found that systems consisting of mixed solvents like PEG – ethanol - water assure high solubility and proper vehiculation in living organisms [6].

The physico-chemical properties of the mixed solvent such as polarity, intermolecular interactions and the ability to form hydrogen bonds and covalent bonds with small molecules ensures the transport and is considered as a "carrier solution". Solvents mixtures can maintain the stability of the chemicals until their interaction with the target molecules in living tissues [1, 7, 8].

In mixed solutions low molecular weight PEG is used. PEG has the chemical structure $HO-(CH_2-CH_2-O)_n-H$ and variable molecular weight depending on the n value of the oxyethylene residue (if $n \geq 4$). Thus, the molecular weight of the PEG can vary between 200-4.000.000 Da. Among them PEG 200 and PEG 400 are in liquid form and are water soluble, while PEG > 600 is waxy solid state. In pharmaceutical industry the low molecular weight PEG is used as a solvent in liquid preparations for oral use as well as in capsules. The higher molecular weight PEG is used as a lubricant and to obtain film-coated tablets.

A mixed solvent consisting of two solvents with different polarity will have an increased capacity to dissolve the powder form drugs. Studies on the increase of the cyclooxygenase-2 inhibitor solubility in mixed solvents attest this fact [6].

For a better therapeutic use of some small proteins, peptides and oligonucleotides one can proceed to their conjugation with PEG, i.e. PEGylation reaction. The most known PEG-ylated proteins are: PEG-asparaginase used in acute lymphocytic leukaemia; PEG-uricase used in gout; PEG-interferon – IFN (i.e. IFN-2α and IFN-2β) which are administered in the treatment of hepatitis C [5, 9].

In the medical field PEG can also be used to prepare laxatives (effectivness by changing environmental osmolality). It is also used as an excipient in different pharmaceutical products [9].

Research on the pharmaceutical use of PEG 400 were performed with human volunteers to assess bioavailability of ranitidine - a product to be used in the treatment of hyperacid gastritis [10].

In food chemistry PEG can be used as antifoaming agent, carrier, emulsifier, glazing agent and thickener. The code of this chemical indicating its use as food additive is E 1521. According to Codex Alimentarius such additives can be added to several food classes, e.g. surface-treated fresh fruits, table-top sweeteners, high-intensity sweeteners, food supplements, water-based flavoured drinks a.o. [4]. The purity criteria for the use of polyethylene glycol (PEG) as a film coating agent for food supplements are given in the Commission Directive EU 67/2010 [11].

In cosmetics PEGs are used in various products such as shampoons, bubble baths, body cleansers, creams, detergents, soaps, toothpastes a.o. as emulsifier, humectant, lubricant [12, 13].

In the technical field is used to getting ink for printers. It is also used to produce electric double layer transistors providing superconductivity [14]. Another use of PEG is the textile industry where it is used as processing and finishing aids, it gives antistatic properties to finished products as well as softness[3]. In wood working operations PEG is a dimensional stabilizer, in paints and inks is a dye carrier, in agriculture is used as an antidusting agent, in tyre manufacturing as lubricant a.o.

Also, PEG can be used in resins synthesis which can immobilize peptides of biological interest or can serve as support for organic synthesis [15, 16].

A study undertaken on triclosan - an antimicrobial agent used in the last three decades in soaps, deodorants, cosmetics and toothpaste followed the evaluation of its anti-proliferative activity on cancer cell culture [8]. For this purpose there were used various single solvents, such as dimethylsulfoxide (DMSO), ethanol (Et-OH), sodium hydroxyde, acetone, and a mixed solvent consisting of 55% PEG 400 and 45% Et-OH. Pursuing selectively the action of the solvent mixture PEG-EtOH it was evidenced differences as compared to the others, i.e. the solvent mixture can create micelle-like structure which may carry the triclosan molecule.

The investigations presented in this paper pursued on an experimental animal model (Wistar strain rats) the effects induced by an organometallic gallium complex, i.e. gallium complex of phosphinobisthiolato P,S,S pincer ligand with the formula :
[PPh4][Ga{PPh(2-SC6H4)2-k3S,S',P}-{PPh(2-SC6H4)2-k2S,S'}]
noted usually C(85). Data regarding the synthesis, molecular structure a.o. of this product were described in a work of Vălean et al. [17]. This complex was dissolved in a mixed solution cotaining PEG.

The procedure using PEG solutions is practiced in solubilizing organometallic compounds of biomedical interest [1, 6, 7]. Co-solvents are considered as "carrier solution" for the compound studied.

In the last decades it was evidenced that many Ga complexes were successfully used in various infectious, inflammatory autoimmune and bone diseases, in the treatment of cancer related hypercalcemia, certain forms of cancer a.o. [18, 19].

2. Experimental

2.1. Chemicals

The gallium complex C(85) received from the Department of Chemistry – Faculty of Chemistry and Chemical Engineering of the "Babeş-Bolyai" University of Cluj-Napoca was dissolved in a solvent mixture (i.e. co-solvents) consisting of: water - ethanol – polyethylene glycol (PEG). In our studies we have used the following substances: ethanol C_2H_5-OH with the molecular weight 46.07 produced by S.C. "P.A.M. Corporation" S.R.L., Romania and polyethylene glycol HO-(CH_2-CH_2-O)$_n$-H with the molecular weight 380-420 and density of 1.13 g/cm^3, produced by Scharlab SL., Pol. Ind. Mas d`en Cisa, Sentmenat, Barcelona, Spain.

2.2. Experimental design

In this study Wistar strain albino rats weighing between 100-120 g were used. Animals were fed with commercial dry pellets and received tap water *ad libitum*. The animals were divided in two series: a morning (m) one – administration of the substances at 7 hrs. a.m. and an evening (e) series - administration of substances at 7 hrs. p.m. Each series consisted of two groups : control (C_p) - injected intraperitoneally (i.p.) with the carrier-solution containing Et-OH 40% : PEG 400 at the ratio 1 : 1.5 (1 mL / 100 g b.w.); experimental group E_{II} - i.p. injection with the solution containing the organometallic gallium complex C(85) solved in the carrier-solution. It is mentioned that the gallium complex was dissolved in the carrier-solution used in previous in vitro studies performed at the "Prof. Dr. I. Chiricuta" Institute of Oncology Cluj-Napoca [unpublished data]. The concentration of the administered gallium complex C(85) in solution to animals of E_{II} groups was 0.25 mg/mL. Each group in each series included 6 animals. The animal groups belonging to the morning series were noted C_p-m and E_{II} -m and those from the evening series C_p-e and E_{II}-e. During experiments - the i.p. administration of solutions - the animals were anesthetized with Anesteran (Isofluorane 99.9%) – manufactured by Rompharm Company Bucharest. By using a special apparatus for narcosis the Anesteran was administered by inhalation to the laboratory animals.

At the end of the experiment, 48 hrs from the administration of the mentioned solutions, the animals were anesthetized again and blood samples were collected for hematological and biochemical analysis. Finally the animals were euthanatized by an Anesteran overdose. Afterwards liver, kidney and brain were collected for some other investigations.

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 [20] and National Governmental Ordinance No.37/30.01.2002 [21]. During the experiments principles of bioethics were respected [22].

2.3. Analytical investigations

Total serum proteins, albumin, non-protein nitrogenous compounds (serum uric acid, creatinine and blood urea nitrogen - BUN) as well as serum magnesium and calcium were determined by the biochemical Spotchem Analyzer and using specific reagent strips manufactured by "Arkray Factory Inc." (Koji-Japan).

Total serum proteins were determined spectrophotometrically based on a reaction with copper sulfate (λ = 550 nm), albumin by the reaction with

bromocresol green ($\lambda = 610$ nm). Globulins can be calculated by the differences to total proteins. Cations Ca and Mg were also determined by using spectrophotometric methods as follows : in case of calcium – reaction with o-cresolphthalein complexone, having blue-purple chromogen effect (λ = 575 nm); in case of magnesium - reaction with the o-cresolphthalein complexone and an EDTA potassium salt, having purple effect (λ = 575 nm).

Uric acid was determined by enzymatic method (uricase in the presence of 4-amino-antipyrine (λ = 550 nm); creatinine – reaction with 3.5 dinitrobenzoic acid ($\lambda = 550$ nm), blood urea nitrogen - reaction of urea (blood) with phtalaldehyde and naphthyl-N`-diethyethylenediamine (λ = 610 nm) – see details in Arkray Factory Inc. Guide.

2.4. Statistical evaluation

All the obtained experimental data were statistically processed, mean values (X) and standard deviations (SD) were calculated. For this purpose the ANOVA (Analysis of Variance) test was used.

3. Results and Discussions

Inorganic and organometallic compounds of Ga were investigated in relation to their possible use for their antibacterial and antiproliferative effects. In the present study there were pursued the effects induced by the organometallic gallium complex C(85) in experimental animals by the evaluation of the chemical parameters of blood serum listed above.

As the amount of the received substance for this research, i.e. the C(85) Ga complex was reduced the experiment was conducted only with a single concentration, pursuing prioritary the homeostatic changes.

That is why in this study we decided to follow the effects of the C(85) Ga complex on total serum proteins and albumin as well as on the main divalent serum electrolytes, i.e. calcium and magnesium. The experimentally obtained values are presented in table 1.

TABLE 1. Homeostasis changes in serum proteins, albumin and two divalent electrolytes

Specifi -cation	n	Proteins (g/dL) X ± SD	Albumin (g/dL) X ± SD	Calcium (mg/dL) X ± SD	Magnesium (mg/dL) X ± SD
C_p-m	6	5.63 ±0.45	3.05±0.45	11.78±0.54	1.90±0.14
E_{II} -m	6	5.56±0.22	2.90±0.32	11.83±0.64	2.01±0.17
ΔX_m		- 0.07	- 0.15	+ 0.05	+ 0.11
C_p-e	6	5.71±0.54	2.96±0.37	11.15±0.45	2.13±0.22
E_{II} -e	6	5.80±0.41	3.15±0.19	11.86±0.42	2.18±0.07
ΔX_e		+ 0.09	+ 0.19	+ 0.71	+ 0.05

Serum proteins are involved in the maintenance of osmotic pressure, pH adjustment, the transport of anions, cations, small biomolecules (e.g. amino acids, fatty acids etc). Thus, the presence of xenobiotic molecules - in this case, the organometallic C (85) complex of Ga - may result in homeostasis changes [23].

It was observed that serum Ga binds to transferrin (the iron-transport protein), its main location is the liver and is excreted by kidneys.

It is known that most plasma proteins, i.e. albumin, fibrinogen, alfa and beta globulins are synthetized in the liver [24]. They have great importance in the osmotic pressure maintenance, blood coagulation, are carriers of phospholipids, metal ions as iron and copper; they participate in the defence mechanism of the organism against diseases a.o. Thus, liver injuries can perturb their serum homeostasis.

The action of inorganic and organometallic gallium compounds on metabolites were described in various papers [18, 25, 26, 27].

Referring to the normal values of proteinemia and albuminemia in rats they are 5.6-7.6 g/dL for total serum proteins and 3.8-4.8 g/dL for serum albumin [28].

In our study, from chronobiological point of view, the values of serum proteins and albumin (see table 1) found in the morning control group C_p-m were lower than those in the evening control group C_p-e.

Evaluating the results of the morning experimental group (E_{II}-m) as compared with those of the morning control group (C_p-m) a slight decrease both in total serum proteins and albumin was revealed. When compared the results of the evening experimental group (E_{II}-m) with those of the evening control group (C_p-e) an increase of serum proteins and albumin was found.

Studying the effects of gallium nitrate on rats Arumugam et al [29] observed an increase of serum albumin from 2.00 to 3.85 g / dL. A research made by Aoki et al [30] on the effects of gallium chloride on rat kidney epithelial tubular cells culture observed an increase in protein synthesis.

It is known that the action of Ga in vivo is more evident on the following metals : Ca, Mg and Zn [18]. In our research there were remarked (see Table 1) in the control groups (C_p-m and C_p-e) a natural evening decrease of Ca and increase of Mg in blood serum – related to the period of administration. In all experimental groups (E_{II}-m and E_{II}-e) an increase of serum Ca and Mg was observed as a result of the Ga complex action administered with a mixed solvent.

In order to evaluate the function of kidneys under the action of the C(85) Ga complex the serum concentrations of creatinine, uric acid and blood urea nitrogen (BUN) were determined. The results are presented in table 2.

As to non-protein nitrogenous compounds when following the values of the control groups C_p-m and C_p-e a natural evening decrease was found. Regarding the effects due to the organometallic Ga complex in all cases a morning and evening increase was observed. Higher values was found for BUN meaning that Ga affects predilectly the urea biosynthesis (ureagenesis).

In a research performed by Arumugam et al. [29] on the effects of Ga nitrate in rats (group of 6 animals) an increase of creatinine and BUN as well as a moderate

decrease of uric acid were observed. These findings indicates that Ga nitrate influences the purine nucleotides metabolism whose end product is uric acid.

TABLE 2. Homeostasis changes in serum non-protein nitrogenous compounds

Specification	n	Uric acid (mg/dL) X ± SD	Creatinine (mg/dL) X ± SD	BUN (mg/dL) X ± SD
C_p-m	6	0.68±0.08	0.61±0.13	16.80±3.52
E_{II} -m	6	0.73±0.14	0.64±0.10	17.60±3.61
ΔX_m		+ 0.05	+ 0.03	+ 1.20
C_p-e	6	0.67±0.08	0.57±0.10	15.80±8.84
E_{II} -e	6	0.72±0.10	0.66±0.05	19.20±7.08
ΔX_e		+ 0.05	+ 0.09	+ 3.40

Research on the effects induced by Ga sulfate on the common carp (Cyprinus carpio) serum parameters was made by Yang et al [31]. The experiment (with 6 animals in group) followed the changes in serum protein, non-protein nitrogenous compounds etc. The authors found a decrease of serum protein concentration and increase of BUN and creatinine. In our study increased levels of serum creatinine, uric acid and BUN were found.

In a context the problem of polymers` use in therapeutics based on the enhancement of studies in this domain has in view not only the advantage of the usage of PEG but also the existence of some disadvantages (e.g. lack of biodegradibility).

Starting from these information researches were intensified on other natural hydophylic polymers like heparin, dextran and chitosan. Recently the interest was focused on biodegradable polymers belonging to the group of amino acids. More frequently the following poly(amino acid)s were studied : poly(glutamic acid) noted PGA, poly(hydroxyethyl-L-asparagine) – noted PHEA and poly(hydroxyethyl-L-glutamine) noted PHEG [32].

Regarding the research on the action of the organometallic compound of Ga it was extended on hematological parameters and on hepatic and renal histological aspects, too.

4. Conclusions

Homeostasis changes under the action of the organometallic Ga complex C (85) in a mixture of solvents with PEG revealed morning decrease and evening increase of serum proteins and albumin. The results of serum calcium and magnesium concentrations showed increases in all groups.

Non-protein nitrogenous substances showed increased values both in the morning and evening animal groups for all the studied parameters. Thus, in case uric acid and creatinine a slight increase was observed. As the increase of BUN it was higher as compared to the other findings which reveals that renal function is influenced by the presence of the Ga compound.

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