

In memoriam
Professor Valer Fărcășan PhD
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Chronobiochemical aspects related to homeostasis changes induced by polyethylene glycol in laboratory animals

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Abstract

Polyethylene glycol (PEG) can be considered as a xenobiotic of food, pharmaceutical and cosmetic interest. In food industry PEG is often used as food additive (E 1521) in water based flavoured drinks (including sport, energy, electrolyte drinks), in table-top sweeteners, in food supplements (see Codex Alimentarius). In the pharmaceutical industry PEG is used as a solvent and in cosmetics as emulsifier, humectant, lubricant.

In a chronobiochemical study (lasting 48 hrs) the effects of PEG as co-solvent and physiological saline on the biochemical homeostasis of laboratory animals (Wistar strain rats) were pursued comparatively. Two animal series (morning - m and evening - m) were constituted. Each series comprised control (C_m and C_e) and experimental (E_m and E_e) animal groups. To Wistar rats of control groups physiological saline and to those of experimental groups PEG solutions were administered intraperitoneally. The concentrations of serum proteins (PRO), albumin (ALB) and the following non-protein nitrogenous metabolites : uric acid (UA), creatinine (CRE), blood urea nitrogen (BUN) were determined. Also, some hematological parameters were pursued : red blood cells (RBC), hemoglobin (HGB), white blood cells (WBC). All the analyses were carried out in the morning and evening. In experimental groups to which PEG solutions were given the results showed for PRO constant values in the morning (i.e. in and decreased values in the evening, while for ALB decreased values both in the morning and evening. As to the non-protein nitrogenous metabolites : a morning decrease and evening increase for UA, morning constant and evening decrease for CRE, morning and evening increase for BUN were revealed. Regarding the obtained values for hematological parameters a morning decrease and evening increase of RBC, HGB and WBC were found. At the studied concentrations the results were statistically non-significant. These experimental data could be predictive in understanding the importance of the controlled quantum of PEG used in foods or pharmaceutical products.

Keywords: chronobiochemistry, PEG, biochemical and hematological homeostasis

1. Introduction

In the last two decades polyethylene glycol (PEG) gained an extensive use in the field of foodstuffs and pharmaceuticals due to its physico-chemical properties and structural features [2, 11, 12].

It is known that PEG along with water, alcohol and glycol is suitable for solubilizing different compounds of nutritional and pharmaceutical interest. Usually the solubilization is made in a mixture of solvents. Systems consisting of mixed solvents like PEG – ethanol - water are able to assure high solubility and proper vehiculation in living organisms [5, 17].

It was shown that the physico-chemical properties of the mixed solvent, e.g. polarity, ability to form not only hydrogen bonds but also 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 [7, 12, 13, 18, 20].

In mixed solutions low molecular weight PEG is used. The chemical structure of PEG is $\text{HO}-(\text{CH}_2-\text{CH}_2-\text{O})_n-\text{H}$ and its variable molecular weight is related to the n value of the oxyethylene residue (if $n \geq 4$) and can vary between 200-4.000.000 Da. In liquid form and presenting water solubility are PEG 200 and PEG 400. 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 further usage of PEG is related to the cosmetic industry. Thus, PEG is present in shampoos, bubble baths, body cleansers, creams, detergents, soaps, toothpastes a.o. as emulsifier, humectant, lubricant [4].

In the technical field PEG is used to getting ink for printers, to produce electric double layer transistors, in wood working operations, in textile industry, in agriculture a.o.

The procedure using PEG solutions is practiced in solubilizing organometallic compounds of biomedical interest [6, 12, 17, 20].

Co-solvents are considered as "carrier solution" for a studied compound.

The aim of the present study was to pursue the action of PEG administered to animals in the morning and evening on some biochemical and hematological parameters in laboratory animals.

2. Materials and methods

Chemicals. In our studies we used : polyethylene glycol (PEG) with the general formula $\text{HO}-(\text{CH}_2-\text{CH}_2-\text{O})_n-\text{H}$, molecular weight of 380-420 and density of 1.13 g/cm^3 , produced by Scharlab SL., Pol. Ind. Mas d'en Cisa, Sentmenat, Barcelona, Spainas well as ethanol $\text{C}_2\text{H}_5-\text{OH}$ with the molecular weight 46.07 produced by S.C. "P.A.M. Corporation" S.R.L., Romania.

Experimental design. Investigations with chronobiochemical specificity were pursued on Wistar strain albino rats weighing between 100-120 g. 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-m, C-e) - injected intraperitoneally with physiological saline and experimental (E-m, E-e) 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.). At the end of the experiment, 48 hrs from the administration of the mentioned solutions, the animals were anesthetized and blood samples were collected for biochemical and hematological analysis. Finally the animals were euthanatized by an Anesteran overdose. There were also excised tissues for other studies.

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 [22] and National Governmental Ordinance No.37/30.01.2002 [23]. During the experiments principles of bioethics were respected [10].

Biochemical investigations. Total serum proteins, albumin, non-protein nitrogenous compounds (serum uric acid, creatinine and blood urea nitrogen - BUN) 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 (λ 610 nm). Globulins can be calculated by the differences to total proteins.

Hematological investigations. Were performed by using an automated “Abacus Junior Vet” type analyzer having a hematology cell counter and implementing the so-called Coulter method. We obtained information on the : total number of red blood cell (RBC), hemoglobin (HGB) and total number of white blood cells (WBC).

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

The performed investigations targeted some biochemical and hematological parameters under

the action of PEG administered in two periods of day (morning and evening).

Our results regarding the concentration of total serum proteins and albumin in the morning and evening are presented in Table 1.

Total proteins (PRO) revealed constant values in the morning and decreased values in the evening. Albumins (ALB) decreased both in the morning and evening.

It is known that most plasma proteins, i.e. albumin, fibrinogen, α - and β -globulins are synthesized in the liver. They have great importance in the osmotic pressure maintenance, blood coagulation, carrying phospholipids and metal ions (e.g. iron, copper), participating in the defence mechanism of the organism against diseases a.o. [7, 8, 9, 15]. Thus, liver injuries can perturb the homeostasis of various blood serum parameters.

In table 2 there are given the obtained values for non-protein nitrogenous metabolites, i.e. uric acid (UA), creatinine (CRE) and blood urea nitrogen (BUN).

A morning decrease and evening increase for UA; morning constant and evening decrease for CRE, morning and evening increase for BUN were revealed.

In anabolic phase of protein metabolism there are formed mainly amino acids and non-protein nitrogenous metabolites. Among these metabolites, there are included: uric acid, creatinine and blood urea nitrogen (BUN).

Table 1. Total serum proteins and albumin fraction

Groups	n	Total proteins (g/dL) X \pm SD	Albumin (g/dL) X \pm SD
C - m	6	5.63 \pm 0.34	3.18 \pm 0.32
E - m	6	5.63 \pm 0.44	3.05 \pm 0.42
Δ X		-	- 0.13
C - e	6	5.90 \pm 0.34	3.26 \pm 0.08
E - e	6	5.71 \pm 0.53	2.96 \pm 0.37
Δ X		- 0.19	- 0.30

Table 2. Non-protein serum metabolites

Groups	n	Uric acid (mg/dL) X ± SD	Creatinine (mg/dL) X ± SD	BUN (mg/dL) X ± SD
C - m	6	0.70 ± 0.16	0.61 ± 0.11	15.66 ± 3.26
E - m	6	0.68 ± 0.07	0.61 ± 0.13	18.00 ± 3.52
ΔX		- 0.02	-	+ 2.34
C - e	6	0.61 ± 0.07	0.63 ± 0.18	16.83 ± 3.43
E - e	6	0.66 ± 0.08	0.56 ± 0.10	21.83 ± 8.84
ΔX		- 0.05	- 0.07	+ 5.00

Uric acid is a catabolic product of purine nucleoproteins metabolism, as well as the de novo synthesis of purines starting from nutrients. Experimental research done on laboratory animals (rats, mice) confirm the increase of serum uric acid values as a consequence of renal injury after administration of metal containing substances (e.g. food xenobiotics and/or pharmaceutical xenobiotics) – Fleck (2001).

Creatinine – another non-protein nitrogen compound - removed also by kidneys in normal conditions showed relatively constant values. Creatine is formed from a non enzymatic reactions

of creatinine in the skeletal muscle. Creatine intake per unit of muscle mass is constant, which explains the relatively constant values with maintaining homeostasis. Initially creatine may result from the amino acids arginine, methionine and glycine. By phosphorylation creatine becomes into an energy source for muscle contractility, being subsequently transformed into creatinine, the form in which they are removed.

Hematological parameters showed are given in table 3 for red blood cells (RBC), hemoglobin concentration (HGB) and for white blood cells (WBC).

Table 3. Main hematological parameters

Groups	n	RBC (•10 ¹² /L) X ± SD	HGB (g/dL) X ± SD	WBC (•10 ⁹ /L) X ± SD
C - m	6	8.76 ± 0.55	14.61 ± 0.54	13.49 ± 6.60
E - m	6	7.83 ± 1.87	12.58 ± 3.32	10.70 ± 3.12
ΔX		- 0.93	- 2.03	- 2.79
C - e	6	9.05 ± 0.80	14.65 ± 1.06	9.09 ± 3.50
E - e	6	9.17 ± 0.45	14.90 ± 1.05	7.66 ± 2.76
ΔX		+ 0.12	+ 0.25	- 1.43

The hematological parameters showed a morning decrease and evening increase of RBC, HGB and WBC.

Red blood cells, hemoglobin concentration and hematocrit are the main indicators of the iron status of the organism and can be modified either by the time-period of collection (morning/evening) or by various nutritional and pathological factors [1, 6, 8]. Data obtained by experiments could be

predictive in understanding the importance of the controlled quantum of PEG used in foods or pharmaceutical products.

Among food related factors one can mention the presence of the mineral micronutrients like Fe, Zn, Cu etc. in foods. In some countries fortification of various foods with iron is compulsory. Regarding the pathological causes one can mention hemorrhages, ferriprive anemia or metabolic disorders of the

porphyrine nucleus biosynthesis. It is known that plack of substances needed for RBC production, side effects of chemotherapy for treatment of leukemia a.o.

In an experiment performed by Sanni et al. [16] on African giant rats the diurnal rhythm of some erythrocytic parameters were studied. The authors found at 12 hrs from the first blood sampling (in the morning) the increase of RBC and HGB while in HCT a decrease was observed.

Diurnal variations of some hematological parameters in healthy adult African giant rats was also studied by Olayemi et al. [14]. In the experiment they used animals divided in 6 groups of 4 animals each and started the blood collection in the morning. The results found for RBC at 12 hrs from the first blood sampling (in the morning) showed a significant decrease. The authors explained the results by the fact that in captivity the African giant rats are more active during the day corresponding with their feeding time.

White blood cells (WBC) or leukocytes have an important role in the defending processes of the organism, e.g. phagocytosis. Regarding the values for leukocytic parameters in African giant rats a decrease of WBC and increase of LYM at 12 hrs interval from the morning blood sampling was reported by Sanni et al. (2000).

In a study performed by Olayemi et al. [16] on African giant rats pursued some parameters related to white blood cells and found that WBC decreased at 12 hrs after the first blood collection (in the morning).

The observed chronobiochemical changes are of interest for nutrition and medicine. Usage of PEG in food and pharmaceutical industry requires knowledge of particular aspects related to it.

In food chemistry PEG can be used as food additive having the code E 1521. PEG can be added to several food classes, such as: surface-treated fresh fruits, table-top sweeteners, high-intensity sweeteners, food supplements, water-based flavoured drinks a.o. (Codex Alimentarius) [25]. Mostly is considered as emulsifier, carrier, glazing agent, antifoaming agent, thickener.

Currently PEG 6000 is permitted as a carrier/carrier solvent for sweeteners. In 2002 the Scientific Committee for Food of EFSA published an opinion on polyethylene glycol (PEG). Thus, 1,4-dioxane, as well as on mono- and diethylene glycol is currently permitted food additives and in proposed use of ethyl hydroxyethyl cellulose (EHEC) in gluten-free bread. Commission Directive EU 67/2010 [24] established the purity criteria for the use of polyethylene glycol (PEG) as a film coating agent in case of food supplements.

Conjugation of some small proteins, peptides and oligonucleotides with PEG, i.e. PEGylation resulted in a better use in therapy [3]. The most known PEG-ylated proteins are: PEG-asparaginase used in acute lymphocytic leukaemia; PEG-uricase administered in gout; PEG-interferon – IFN (i.e. IFN-2 α and IFN-2 β) in the treatment of hepatitis C [2, 19]. PEG can be used also as excipient in various pharmaceutical products or to prepare laxatives.

4. Conclusions

1. Animals of experimental groups receiving PEG solutions showed for PRO constant values in the morning and decreased values in the evening. Regarding ALB values there were decreased both in the morning and evening.
2. Non-protein nitrogenous metabolites revealed : a morning decrease and evening increase for UA; morning constant and evening decrease for CRE, morning and evening increase for BUN.
3. The hematological parameters showed a morning decrease and evening increase of RBC, HGB and WBC.
4. All the found changes at the studied concentration were statistically non-significant, attesting that in can be used both in food supplements and medicinal products.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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