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Oxidative Modifications of Proteins in Intact Rats and in Rats Exposed to an Electrostatic Field are in Strict Dependence on the Phases of the Moon

Hayk A Harutyunyan*

Laboratory of Biochemical and Biophysical Investigations, Scientific-Research Center, Yerevan State Medical University after M. Armenia

***Corresponding author:** Hayk A Harutyunya, Laboratory of Biochemical and Biophysical Investigations, Scientific-Research Center, Yerevan State Medical University after M. Heratsi, Armenia

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ABSTRACT

The aim of this study was to determine the dynamics of fibrinogen carbonylation in rats and the influence of the external electrostatic field of higher tension (200 kV/m) on this parameter. The correlation between the level of fibrinogen oxidation and moon cycles in intact rats has been shown. Moreover, the effect of external electrostatic field on the carbonylation of fibrinogen in plasma also depended from the moon phases.

Keywords: Carbonylation of Fibrinogen; External Electrostatic Field; Moon Phases

Introduction

An Electrostatic Field (ESF) is the force field created between electric charges that are fixed in space and do not vary with time. External ESF is a widely distributed physical factor which has both natural and human made origins. Different exertions of ESF were detected in human environment. The primary cause of the field is the charge separation that occurs between the Earth and the ionosphere (König, et al. [1]). The field near the surface typically has fair weather strength of about 130 V/m (Dolezalek [2]). As the cloud approaches, the field at ground level may first increase and then reverse with the ground becoming positively charged. Fields of 100 V/m to 3 kV/m may be observed during this process (Grandolfo, et al. [3,4]). The next common cause of human exposure is charge separation as a result of friction. Charge potential of several kilovolts can be accumulated while walking on non-conducting carpets, generating local fields of up to 500 kV/m. The handling and treating of plastics can produce ESF strengths of 100 - 300 kV/m near the body. Direct current electric transmission lines (500 kV) can produce ESF of up to 20 kV/m (EC [5]). Hence, nowadays artificially increased background of ESF is obvious and the biological activity of this factor is the subject of different investigations. Official reports of WHO and other responsible organizations speak in favor of little biological evidence to suggest any adverse effect of ESF on human health. Few animal studies that have been carried out also appear to have yielded no data supporting adverse effects of ESF (Grandolfo, et al. [3,4]). In another document was reported a fragmentary and coherent approach to investigate the biological consequences of exposure of ESF, and in many areas the data are insufficient to draw conclusions regarding the possibility of health effects, especially following chronic exposure (NRPB [6]).

Data about ESF biological effect provided in scientific literature are controversial and sometimes mutually exclusive. A review by (Kowalczuk, et al. [7]) concluded that the few published animal

studies about static electric field effects provided no evidence of any adverse health effect. On the other hand, Lott and McCain (Lott, et al. [8]) in earlier work showed increased EEG activity and decreased posterior hypothalamic activity during exposure of ESF of 10 kV/m. Marino (Marino, et al. [9]) exposed groups of rats to static electric fields at 0.3 to 19.7 kV/m for 30 days and detected changes in the serum protein fractions. Alterations in the blood plasma and serum proteome at rats exposed to ESF (200 kV/m) were shown also in our investigations (Harutyunyan, et al. [10]). There is increasing experimental evidence that externally applied ESF exerts various effects on oxidative processes and antioxidative defense systems (Seyhan, et al. [11,12]). In general, the inconstancy of the data, shown in several investigations, is at the bottom of the data ambiguity on biological activity of the external ESF (Grandolfo, et al. [3,4,7]). So, there is a question about the reasons for the inconsistency of these data. Current article is addressed to the inconstancy of the data on plasma fibrinogen carbonylation in rats exposed to ESF. Fibrinogen is known as one of the plasma proteins most susceptible to oxidative modification (Stikarova, et al. [13]). Hence, the level of oxidative modification of fibrinogen can reflect the overall status of pro-/antioxidant homeostasis. Literature data on the fibrinogen carbonylation in plasma of intact rats vary widely (0.8-9.0 nmol/mg) (Harutyunyan, et al. [12-14]). The same is true for the data on carbonylation of total plasma protein. The literature data on this parameter in intact rats varies from about 0.1-0.5 to about 5-8 nmol/mg (Kennedy, et al. [15-18]).

Numerous reasons can determine the difference of plasma proteins carbonylation and the ambiguity of data on biological activity of ESF. In this regard, our attention was drawn to the notice from the article of Zimecki M, titled "The lunar cycle: effects on human and animal behavior and physiology". He writes, "The lunar cycle exerts effects on laboratory rats. It is suggested that melatonin and endogenous steroids may mediate the cyclic alterations of physiological processes. The release of neurohormones may be triggered by the electromagnetic radiation and/or the gravitational pull of the moon" (Zimecki [19]). Indeed, the moon has a significant impact on a variety of physiological processes in the body (Tarlow, et al. [20-22]). In particular, the influence of moon cycle might be one of the reasons affecting the state of pro-/antioxidant homeostasis in rats and also might has an impact on biological activity of investigated ESF. In the current paper the data of investigations of the influence (*in vivo*) of ESF (200 kV/m) on the carbonylation of fibrinogen in rats are provided. Several independent series of experiments was carried out in the period of 2011-2013. Both male and female animals were used in the investigations.

Materials and Methods

Adult albino outbred male rats weighing 163-191g were randomly divided into 4 groups (2 examined and 2 control groups). Rats from examined groups were exposed to ESF (200 kV/m) during 1 h - from 900 to 1000 (acute exposure) and for 6 succeeding days 6 h a day - from 900 to 1500 (chronic exposure). Rats from control groups were subjected for the same time intervals to sham-exposures, during which they stayed in identical conditions, excluding the influence of ESF, when no voltage between electrodes was generated. Sham-exposed controls were used for acute and for chronic exposures separately. During the exposure animals were placed in special plastic cages (5 animals in one cage), which did not disturb applied ESF and allowed rats to move freely. Animals were kept in optimal environmental conditions (constant humidity of air: 60% and temperature: 21^0C, 12 h light-dark cycle). They were fed with routine laboratory food for rodents as well as given unlimited access to water.

ESF was generated using locally constructed experimental exposure system (Artsruni, et al. [23]). E = 200 kV/m was generated between 2 round electrodes (diameter = 1.5 m) placed on the distance of 30 cm from each other. In order to obtain the ESF of 200 kV/m strength, on the upper electrode the constant negative potential of 60 kV was applied, while the lower electrode was grounded. Blood samples were collected during 10-15 min after the end of short- and long-term exposures to ESF or sham-exposures (controls). Blood was obtained by cardiac puncture using polypropylene syringes (B. Braun Melsungen AG). Blood was immediately centrifuged (10 min at 500 × g). Obtained plasma was additionally centrifuged at 4000 × g for 10 min and the supernatant was used for the measurements.

Fibrinogen was isolated from citrated plasma by the sodium sulfate precipitation (Na_2SO_4, 10,6 % final) (Cohen [24]). Carbonylation was measured by the method of Levine (Levine, et al. [25]) using the reaction with 2,4-Dinitrophenylhydrasine (DNPH). Spectrophotometer Hitachi 150-20 UV-VIS (Japan) was used for the absorbance measurements. The protein carbonyl content was expressed in terms of nmol/mg of protein.

Statistical analysis of the results was done using the statistical functions of the GrafPad InStat software (GraphPad Software, Inc., San Diego California USA, www.graphpad.com). Two independent groups were compared using Mann-Whitney U test. All data were expressed as mean ± standard error of mean (M±m). P-value < 0.05 was considered statistically significant.

Results and Discussion

Obtained data on fibrinogen carbonylation in rats exposed to external ESF were far from being unambiguous. Results of these experiments are presented in Figure 1. It is clearly shown that obtained data on fibrinogen carbonylation in rats after ESF exposure vary from one experiment to another independently from ESF exposure time. Hence, in May 2011 short term action of ESF brought to substantial increase of fibrinogen oxidation at male rats. On the other hand, experiments carried out in Nov 2012 (female) and in Apr 2013 (male) resulted with diminished values as compared with control. In Dec 2012 (female) we did not detect any significant effect of the short term ESF exposure on fibrinogen carbonylation (Figure 1A). The situation was the same in studying of long term ESF exposure (6 days, 6 hours daily). In the part of experiments (Oct 2011 and Dec 2012) ESF had a positive effect on the oxidative modification of fibrinogen and in the next experiments (Nov 2012 and May 2013) the negative influence of ESF was detected (Figure 1 B). It is worthy of notice that the level of fibrinogen carbonylation vary widely (3 - 12 nmol/mg) in control group also.

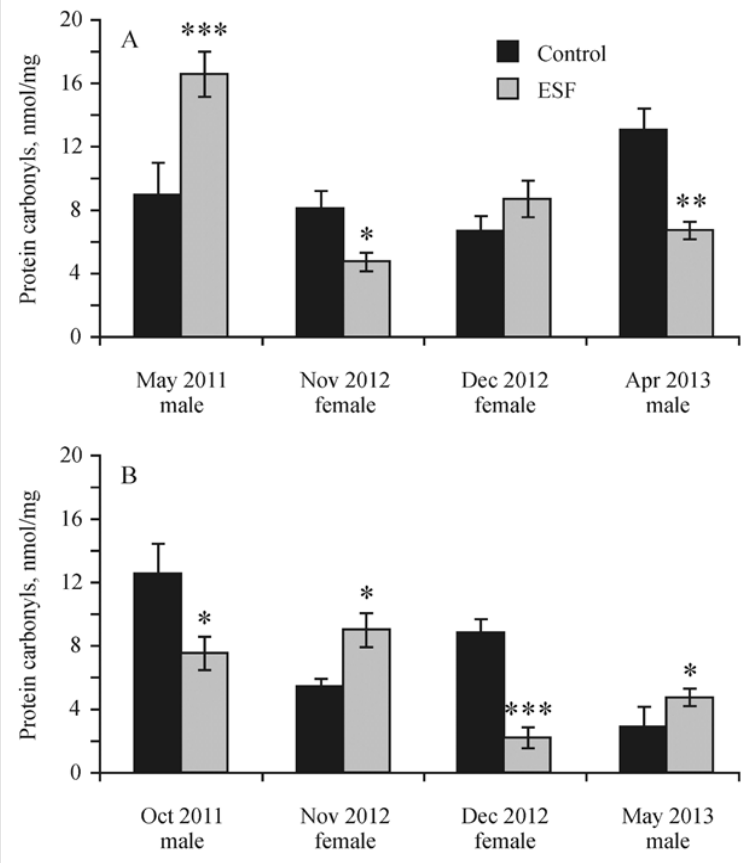

Figure 1: Fibrinogen carbonylation in rats exposed to external ESF (200 kV/m). Data of experiments carried out during 2011 – 2013. A – after short term ESF exposure (1 hour); B – after long term ESF exposure (6 days, 6 hours daily).

The Following Questions Were Arisen from the Data Provided Above

Which external factor governs the observed fluctuations of the fibrinogen carbonylation in the control (intact) animals? 2 - What is the reason of the data inconstancy in the research of ESF effect (*in vivo*) on fibrinogen carbonylation? With the goal to clarify these questions the pack of experiments had been initiated in Oct-Dec 2012. The time dependence of the oxidative modification of fibrinogen was investigated during this period. The carbonylation of fibrinogen was measured at certain intervals in intact and ESF exposed rats. Six animals were assessed at every point. Blood was collected by cardiac puncture twice from every animal (after necessary recovery period - 2 weeks). Thus, 66 female rats (173 g on average) were used in the study and 132 measurements were done in all. The wave curve of the dynamics of fibrinogen carbonylation was observed at intact (control) rats (Figure 2A). The maximal values of studied parameter in control rats were detected on 31 Oct (9.40±0.52 nmol/mg), 15-26 Nov (8.47±0.63 nmol/mg) and on 12 Dec (10.63±1.41 nmol/mg). On the 05 Nov (3.72±0.28 nmol/mg) and 04 Dec (4.91±0.55 nmol/mg) the minimal values of fibrinogen carbonylation in intact animals were observed.

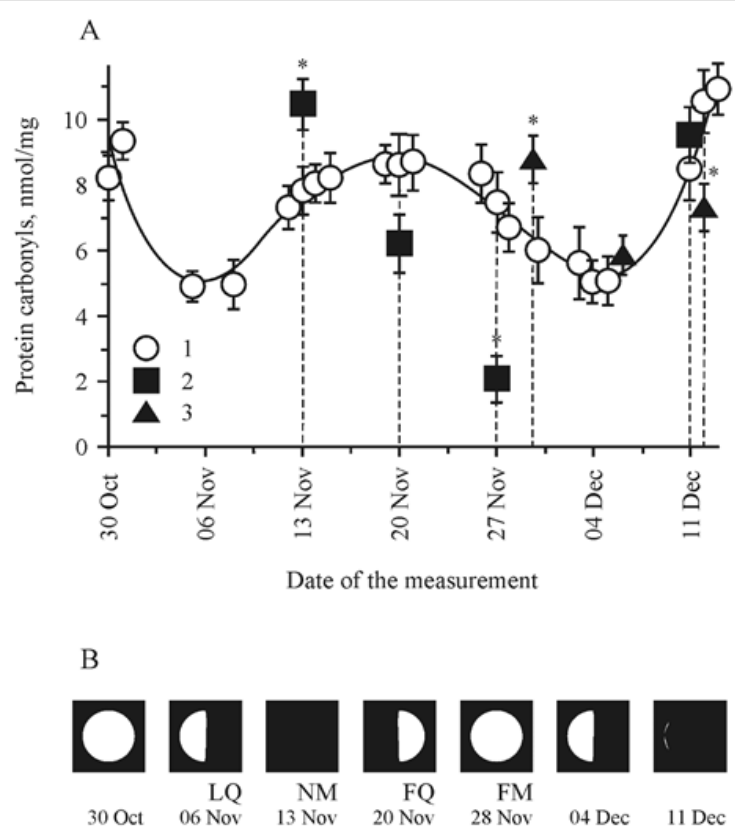

Figure 2: Fibrinogen carbonylation and the moon phases. A – The time dependence of the fibrinogen carbonylation in intact rats and in animals exposed to external ESF (200 kV/m). Data of experiments carried out during Oct-Dec 2012. 1 – control (intact animals); 2 – after short term ESF exposure (1 hour); 3 – after long term ESF exposure (6 days, 6 hours daily). * $P<0.05$; n=6. B – moon phases for the study period (Oct-Dec 2012). LQ – Last Quarter; NM – New Moon; FQ – First Quarter; FM – Fool Moon.

The values of fibrinogen carbonylation in the control rats oscillated in the substantial ranges in dependence from the measurement date. It was evident that the "wave-length" of this curve equals to about 28-30 days. It seems very similar to duration of the moon cycle. Thus, obtained data on fibrinogen carbonylation were compared with the lunar calendar for mentioned period of time. Data on moon phases were obtained from the Newsletter of Hodgson Observatory [26]. Hence, fibrinogen carbonylation values in intact rats were maximal during First Quarter (FQ) of moon phase (Figure 2B). Then, this parameter dropped down which was coincided with the Fool Moon (FM). At the Low Quarter (LQ) we observed minimal values of fibrinogen oxidation. Next, enhancement of this parameter was shown for New Moon (NM) period. Coincidence of fibrinogen carbonylation data with moon phases seems highly plausible. Above it, the effects of artificially applied ESF (200 kV/m) were manifested and were specific for the certain moon phases only. In particular, we did not observe any significant effect of ESF on the fibrinogen carbonylation of rats at LQ and FQ phases.On the other hand, at NM and FM the influence of ESF was highly expressed on the oxidative modification of fibrinogen.

Moreover, ESF exposure at NM and FM resulted with opposite effects. While long term ESF exposure (6 days, 6 hours daily) brought to enhanced fibrinogen carbonylation at NM, the diminished oxidation was shown at FM period. The short term ESF exposure (1 h) expressed with reverse results, i.e., enhancement of fibrinogen carbonylation at the period near to FM and diminishing at the period near to NM was observed. Inspite of such correlation between the moon cycle and the oxidative modification of proteins was not described earlier; there are a lot of data evidencing effect of the moon on various biological processes including reproductive behavior, birth rate, blood loss etc. (Abell, et al. [27-29]). Observed correlation seems to be highly speculative. However, further studies need to prove the statement described above. This might mean that the moon phases as an external factor should be taken into account during designing of the biological experiment [30].

Conclusion

During the period of the study of fibrinogen carbonylation in ESF exposed rats (2011-2013) the inconstancy of the data was observed. In further investigations the correlation between the level of fibrinogen oxidation and moon cycle in intact rats has been shown. The dependence from the moon phase was detected also for the effect of the external ESF on the oxidative modification of fibrinogen in rats.

Declaration of Interest

The authors report no declarations of interest.

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