UNIVERSIDAD DE GRANADA

Grupo de Investigación CTS-101:
"Comunicación Intercelular"

BIOCHEMICAL EVALUATION OF THE NEUTRALISATION OF THE EXTREMELY LOW FREQUENCY MAGNETICS FIELDS IN HUMANS

Prof. Germaine Escames
D. Francisco Ortiz

Biomedical Research Center
Health Sciences Technology Park
University of Granada
Avenida del Conocimiento, s/n
18100-Armilla (Granada)
Tel.: +34 958 241000 ext. 20197
Fax: +34 958 819232
e-mail: gescames@ugr.es

Pranan Technologies
Tel. 948 20 32 25

1. EXPERIMENTALS GROUPS

The study has been performed in 20 healthy subjects, aged 30-50 years, with no medication and without neurological or psychiatric disorders. All volunteers use cellular phones regularly and all of them signed an informed consent.

For this study, the volunteers have been provided by three devices of the Pranan Technologies Company.

2. BIOCHEMICAL ANALYSIS

Plasma determinations:

-Cortisol
-Melatonin
-Lipid peroxidation
-Nitric oxide
-Pro-inflammatory cytokines: IL-1β, IL-2, IL-5, IL-8, IL-6, INFγ, TNFβ
-Anti-inflammatory cytokines: IL-10

Erythrocytes determinations:

-Disulfide glutathione (GSSG)/glutathione (GSH) ratio
-Glutatione peroxidase
-Glutathione reductase
-Superoxide dismutase

Urine determinations:

-6-sulfathoxymelatonin

3. EXPERIMENTAL PROTOCOL

Blood samples were obtained from the antecubital vein in all subjects at 9:00 am, before and one month after using the three devices. Blood samples were collected into vacutainers containing EDTA-K2 and centrifuged at 3,000 g for 10 min at 4 °C. Plasma aliquots were separated and frozen at -80 °C until the biochemical assays were performed. Erythrocytes were separated, washed twice with cold saline, and frozen at −80 °C. On the day of the experiment, washed erythrocytes were hemolized in phosphate buffer (10 mM sodium phosphate,1 mM EDTA-Na2, pH 6.25), deproteinized with ice-cold 10% trichloroacetic acid, and centrifuged at 20,000 g for 15 min. Supernatants were then used for the measurements. The urine was collected between 12 pm and 8 am. The diuresis was measure and aliquots of 10 ml were frozen at 80 °C until their use for the determination of 6-sulfathoxymelatonin.

4. OBJECTIVES

1º. To evaluate the effects of the simultaneous use of three devices developed by Pranan Technologies (Armonizador Pranan 8-R-5 Relax, Vitalizador 8-V-11 Pranan, Neutralizador Pranan Phone) on markers of extra- and intracellular oxidative/nitrosative stress following the attached protocol.
2º. To correlate the results of the objective 1 with physical stress, through the determination of plasma cortisol, as well as cytokines.
3º. To correlate the above data with the activity of the endogenous antioxidant system, mainly constituted by the glutathione redox cycle and melatonin.

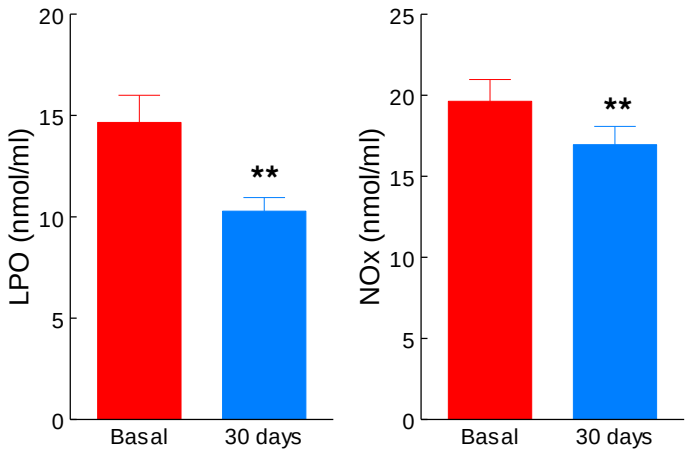
5. RESULTS

5.1. Markers of oxidative stress

The following parameters were analyzed: extracellular levels of lipid peroxidation (LPO) and nitrites (NOx) in plasma, and intracellular ratio of disulfide glutathione (GSSG)/glutathione (GSH) and the activities of the glutathione redox cycle enzymes, glutathione peroxidase (GPx) and reductase (GRd) in erythrocytes.

5.1.1. Markers of extracellular oxidative stress

Malondialdehyde (MDA), the end product of lipid peroxidation is a highly toxic molecule implicated in a range of pathologies. In turn, nitric oxide, which was evaluated as the levels of nitrites+nitrates (NOx), is a free radical gas that has beneficial properties as a mechanism of cellular repair at night, but it is extremely toxic at high concentrations. It is related to all degenerative diseases and inflammatory processes.



Figures 1: Levels of extracellular oxidative stress markers in the studied subjects before (Basal) and after 30 days using the devices of PRANAN Technologies.

The changes in plasma LPO and NOx levels are illustrated in Figure 1. Compared with the control group, after 30 days using PRANAN Technologies devices, the levels of oxidative stress decrease significantly. One month using the devices was enough to reduce LPO ($P<0.01$) and NOx ($P<0.01$) in plasma. The data obtained in this study provide the first evidence to support the beneficial effect of these devices against oxidative stress.

5.1.2. Markers of intracellular oxidative stress

The cell contains important antioxidant machinery. Among them, there is a group of enzymes specially involved in detoxifying the cell from free radicals. These constitute the enzymes involved in the control of the glutathione redox cycle.

Figure 2 shows the levels of the erythrocytic GSH and GSSG, and the GSSG/GSH ratio. The devices of PRANAN Technologies reduced the GSSG/GSH ratio in erythrocytes after 1 month of use ($P<0.05$), mainly due to a reduction in the GSSG levels and a slight increase in the GSH ones in red blood cells. Total glutathione (Gtotal) did not change along the study. Taking into account that the GSSG/GSH ratio is the best index of the intracellular redox status, and that this ratio in erythrocytes reflects well the situation in other cells of the body, our data confirms that 30 days of devices implementation significantly reduced the free radical damage to the cells.

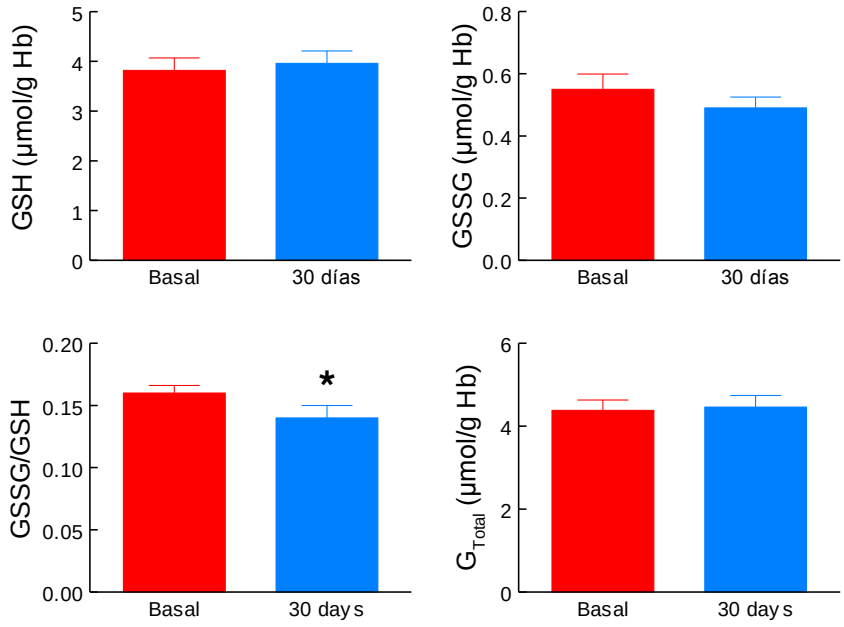


Figure 2: Intracellular changes of the glutathione system in red blood cells before and 30 days after of using the PRANAN's devices.

The glutathione peroxidase (GPx) is an enzyme that converts the hydrogen peroxide into water, with the simultaneous oxidation of one GSH molecule. GPx increased significantly ($p<0.01$) after 30 days of Pranan's devices use (Figure 3), which reflects an improvement of peroxide detoxification. The glutathione reductase (GRd) is the other enzyme of the glutathione redox cycle, responsible for the recovering of the GSH levels from GSSG. It is

essential to maintain the pool of intracellular GSH. This enzyme increased significantly with the use of Pranan's devices (Figure 3, $p<0.01$). Consequently, the efficiency of the glutathione redox cycle is significantly improved by the use of these devices.

In the case of superoxide dismutase (SOD), its antioxidant activity is related to the reduction of superoxide anion, which is dismutated to hydrogen peroxide. The decrease of the activity of this enzyme reported in this study ($p<0.05$), reflects a lower production of superoxide anion and, therefore, a decrease of the requirements of this enzyme. Therefore, SOD changes reflect a lesser degree of oxidative stress after Pranan's devices use.

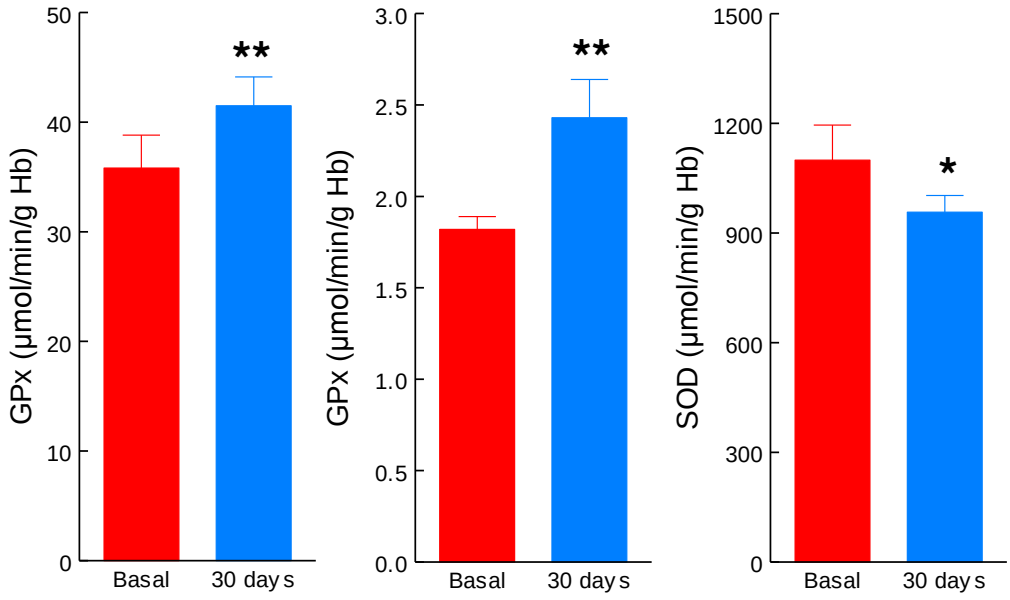


Figure 3: Activities of the intracellular antioxidative enzymes in red blood cells before and 30 days after the use of Pranan's devices.

5.1.3. Melatonin: an antioxidative and anti-inflammatory molecule

Melatonin is a hormone that combats free radicals and reduces inflammation, increases the capacity of the immune system, reduces the risk of getting cancer, heart, and neurodegenerative diseases, and improves sleep at night. These actions have an important application to prevent aging and the aging-related diseases. Melatonin is a stress hormone, and it increases in conditions such as oxidative stress and inflammation. In our study, we did not observe significant changes in the levels of plasma melatonin along the study.

This is a typical behavior of melatonin, which generally reflects that the hormone is quickly consumed by its antioxidant activity. Thus, consumed melatonin is quickly replaced by "de novo" melatonin synthesis and, thus, its plasma levels remains unchanged. Anyway,

the preservation of its levels reflects an appropriate functioning of its role as the main component of the endogenous antioxidant system.

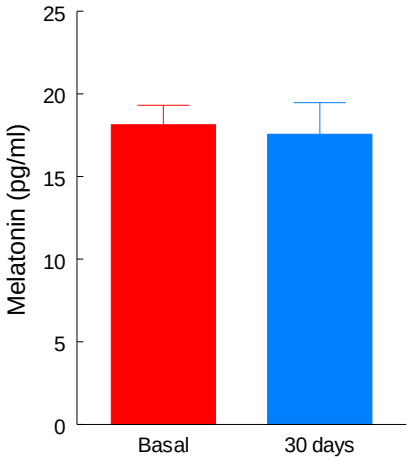


Figure 4: Plasma levels of melatonin before and after 30 days of using the Pranan's devices.

5.2. Markers of inflammation

For this purpose, we used two main markers: 1) the plasma levels of nitrites (NOx), which reflect the production of nitric oxide and they were included in the figure 1, and 2) the levels of pro-inflammatory and anti-inflammatory plasma cytokines.

In the first case, the results demonstrated a significant reduction in the inflammatory response which is reflected in the decrease of the levels of nitrites (Figure 1). There is, therefore, a reduction of the chronic inflammatory state in these subjects.

In relation to the pro-inflammatory cytokines [(interferon γ (INF-γ), interleukin 1β (IL-1β), interleukin 2 (IL-2), interleukin 8 (IL-8), and tumor necrosis factor β (TNF-β)], as well as the anti-inflammatory cytokines [interleukin 6 (IL-6) and interleukin 10 (IL-10)], we can see that the basal levels of these cytokines are within the normal range. We didn't find any significant changes after the 30 days except for INF-γ, which decreased significantly after 30 days ($P<0.05$), speaking in favor of a decline in the inflammatory status. Together, reduction in both NOx and INFγ levels support the antiinflammatory activity of the PRANAM devices. These results show an improvement in the overall state which is subjected to a lower burden inflammatory stress.

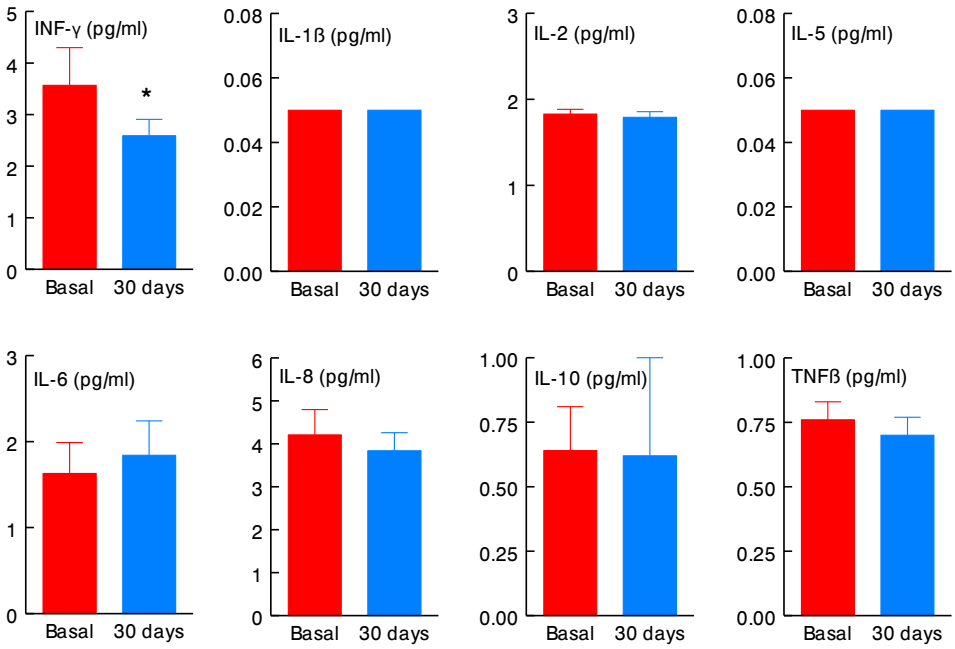


Figure 5: Plasma levels of pro and anti-inflammatory cytokines before and after 30 days of using the PRANAN Technologies devices.

5.3. Markers of chronic stress

For this purpose, plasma levels of cortisol and urinary excretion of 6-sulfatoxymelatonin were determined.

Plasma levels of cortisol, measured at 9 am before and after one month using the Pranan's devices, did not show significant changes (Figure 6).

Regarding the urinary excretion of melatonin, a tendency to increase at the end of the experiment was observed, although no statistical significance was found.

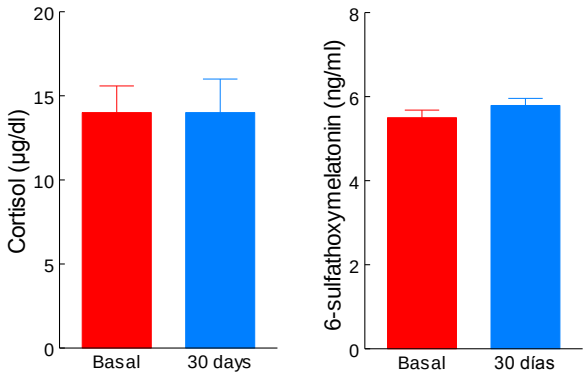


Figure 6: Plasma levels of cortisol (left) and 6-sulfathoxymelatonin (right) before and 30 days after using the Pranan's devices

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6. CONCLUSIONS

The results of this work certainly demonstrate that the devices of PRANAN Technologies (Armonizador Pranan 8-R-5 Relax, Vitalizador 8-V-11 Pranan, and Neutralizador Pranan Phone) used in this study, significantly reduced markers of oxidative stress and inflammation in normal population, correcting toward a better physiological status of health.

Accordingly, the devices of PRANAN Technologies provide the cell with additional protection against a range of harmful electrophiles produced during oxidative stress, which helping in the maintenance of a satisfactory health condition.

It must be taken in mind that the results here reported have been obtained in a population of normal subjects, with no diseases or drug therapy. Thus, in subjects who present an oxidative stress/inflammatory condition, the beneficial effects of the Pranan's devices would be more striking.

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Germaine Escames
Professor of Physiology
University of Granada