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MARKERS OF ENDOGENOUS INTOXICATION AND OXIDATIVE STRESS IN PATIENTS WITH OPIOID DEPENDENCE SYNDROME

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***Iliya Bykov*[1]✉, *Konstantin Popov*[1], *Dmitry Lubchenko*[1,2], *Munya Popova*[2], *Fedor Filippov*[1], *Anzhela Stolyarova*[1], *Yana Denisova*[1], *Dmitry Domenyuk*[3]**

[1] *Kuban State Medical University, Krasnodar*
[2] *Regional Narcological Dispensary, Krasnodar Region*
[3] *Stavropol State Medical University, Stavropol*

✉ *ilyaMB@ksma.ru*

ABSTRACT — Aim of study is to evaluate changes in fluorescence indicators that reflect the endotoxicosis and oxidative stress status in patients with opioid dependence syndrome through treatment.
Materials and methods. The study involved 28 patients with opioid dependence syndrome and 20 healthy persons. Fluorescence parameters reflecting the accumulation of proteins oxidative modification products were determined in the blood plasma.
Results and discussion. At the stage of admitting the patients to the hospital, the blood plasma was found to contain increased levels of FLOPs and bityrosine residues by 18–30% against 12–20% reduction of the intensity of intrinsic and probe fluorescence of proteins. At the same time, upon the completion of the therapy course (15–17 days) all the studied parameters returned to the values those in the control group, except for the level of bityrosine residues, which remained 20% above that in the control. This may be accounted for by the process of updating plasma proteins and the greater stability demonstrated by bityrosine molecules.
Conclusion. FLOPs and tyrosine residue identification can be used for laboratory monitoring of early response to the therapy as well as for a long-term efficiency follow-up in patients with opioid dependence syndrome.

KEYWORDS — dependence syndrome, opioids, drug addiction, laboratory diagnostics, oxidative stress, blood plasma fluorescence.

INTRODUCTION

To date, a growing dependence on psychoactive substances presents an unfavorable trend of an increase in related morbidity and mortality. According to the United Nations office on drugs and crime, up to 272 million people have tried illicit substances at least once in 2010, and about 200,000 people die annualy from drug-related issues [1]. One of the most promising laboratory markers in addiction-treatment practice are the indicators of the intensity of the inflammatory response, as well as indicators of vascular damage, which also include oxidative stress markers [2, 3]. The link between chronic opioid exposure and oxidative stress has been shown in experimental studies, which in particular focus on the development of mitochondrial dysfunction, an increased production of the reactive oxygen species in the mouse brain mitochondria, lipid peroxidation products, and the presence of carbonyl protein residues along with a decrease in the glutathione concentration [4]. The data from clinical studies also indicate on a significant role that oxidative stress plays in etiology of the dependence syndrome. At the same time, we have not found enough explanation on the use of markers of free radical activity and the functional status of the antioxidant defense system in laboratory monitoring and prediction of drug pathology [5, 6].

Aim of study

to evaluate changes in fluorescence indicators that reflect the endotoxicosis and oxidative stress status in patients with opioid dependence syndrome during the treatment.

MATERIALS AND METHODS

The study was carried out using biological material (peripheral blood) from patients with opioid dependence syndrome at the Krasnodar Region Addiction Clinic. There were a total of 28 patients and a control group comprised 20 relatively healthy individuals. Blood was collected from the patients by the first day after admission and prior to their discharge. The average treatment length was 15–17 days.

The treatment was aimed at correcting the major mental disorders. In blood plasma, we measured the intensity of intrinsic fluorescence of tryptophanyl proteins (excited by light at 280 nm and registering light emission at 330 nm), as well as the intensity of fluorescence (excited by light at 380 nm and registering light emission at 490 nm) of the 1,8-ANS (1-anilin-8-naphthalenesulfonic acid) probe when binding to plasma proteins, mainly albumin [7]. To assess oxidative damage, we determined the level of fluorescent products of oxidative damage of proteins (FLOPs), bityrosine,

as well as identified the fluorescent parameters typical of plasma proteins non-enzymatic glycation products (AGE). FLOPs measurements were done at the fluorescence excitation/emission wavelengths of 320/420, 360/420, and 400/475, while the parameters were marked by the excitation wavelengths (FLOP 320, FLOP 360, and FLOP 400) [8]. The content of bityrosine residue was also evaluated employing the fluorimetric method with the registration at the emission wavelength of 410 nm while excited by light at 310 nm [9]. The protein glycation products were identified under conditions of excitation by light with a wavelength of 370 nm, while recording the emission of light at a wavelength of 404 nm [10,11]. The laboratory part of the study was performed with the SM2203 spectrofluorometer (Solar, Belarus). All the measurements were carried out in a temperature-controlled cuvette at 25° C. The study was approved by the independent ethics Committee at Kuban State Medical University (Protocol #58 of 11/12/2017).

The statistical data processing was performed with AnalystSoft Inc., StatPlus statistical analysis software, Version 7 (see www.analystsoft.com/ru/). To compare the values obtained from the control group and the comparison group, the nonparametric Mann-Whitney test was employed, as well as the nonparametric Wilcoxon test, which was used to compare the values for the patients from the experimental group prior to, and after, the treatment. The differences were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

The study has revealed that the patients with opioid dependence syndrome had a statistically significant growing content of products of oxidative damage of proteins which was detected through fluorescent methods. At the point of admission to the addiction clinic, the FLOPs levels exceeded the values of the respective indicators in the control group by an average of 18–30%. The blood plasma content of FLOP 320 in patients with opioid dependence syndrome exceeded the control values of the same factor by 24%, whereas the level of FLOP 360 went up by 18%, and the FLOP 400 level — by 30%.

An evaluation of the bityrosine residues content at admission also was observed at a 20% increase in the level of this oxidative protein damage product in the blood plasma. Measuring the level of accumulation of protein glycation endproducts which is a common method of controlling hyperglycemia in patients or when modeling diabetes mellitus revealed a small yet statistically significant increase in this value by 8%. This observation, however, offers an explanation to separate identification of oxidative modifications and glycation products by fluorescent methods, despite the seemingly low specifics of these methods. This result was not unexpected due to the well-known fact of the oxidative stress status in patients with drug dependence; the determination of proteins non-enzyme glycation fluorescent products seemed plausible, too, in view of this process acceleration not against hyperglycemia only, yet also in view of its strengthening during oxidation and other protein damage.

The identification of the intensity in the plasma proteins intrinsic and probe fluorescence was aimed at detecting their conformation disturbance, which can develop due to the same oxidation modifications or due to endotoxicosis, since protein molecules interact actively with other molecules, including inorganic ions, low-molecular organic compounds and peptides. The outcomes of studying the intrinsic fluorescence of tryptophan residues and the 1,8-ANS probe fluorescence in the blood plasma in patients with opioid dependence syndrome featured initially reduced values in their intensity — by 12% and 20%, respectively.

The values, obtained after therapy and analyzed through this study revealed a partial recovery, which implied an increase in the level of tryptophanyl intrinsic fluorescence and the intensity of 1.8-ANS fluorescence to the control values of the corresponding indicators. The level of FLOPs also increased at the stage the patients were discharged from the clinic, and reached normal values. Given that, the content of tyrosine residues remained initially increased by 20% after 15–17 days of therapy as well. Such results may be due to a fairly long observation period — over 2 weeks; this period is enough to ensure almost complete renewal of serum albumin molecules (average life span — 2–3 weeks) — the major protein of blood plasma, which determines the native features of the biofluid proteins fluorescence. At the same time, the bityrosine molecule is quite stable and persists through the said period.

CONCLUSION

The study outcomes demonstrate the potential of evaluating fairly simple blood plasma fluorescent parameters enabling to examine the severity of oxidative damage to proteins. Parameters like FLOPs and bityrosine residue levels can be used for laboratory monitoring of metabolic disorders in patients with opioid dependence syndrome, as well as for monitoring the effectiveness of therapy. Given the different types of changes in the proposed markers, they may prove useful both as a short term mode — for assessing early response to therapy, and as a long-term one — to assess therapy or rehabilitation compliance.

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