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**Article name:** **Антиоксидантное и мембранопротекторное действие СКЭНАР-терапии при опийной наркомании**

## **Antioxidant and membrane protecting effects of SCENAR-therapy in treating opium addiction**

### **Summary:**

The research showed that including SCENAR-therapy into the complex treatment of opium-addicted patients greatly increases the produced therapeutic effect and decreases the intensity of oxidative stress. Such an effect is achieved by stimulating antioxidative enzymes - superoxide dismutase and catalase in erythrocytes – and inhibiting the intensity of free-radical processes in the blood. Decreasing the level of lipid peroxidation in erythrocyte membranes provides increase in stability and normalization of erythrocyte membrane structure.

**Key words:** opium-addiction, SCENAR-therapy, free-radical processes, antioxidant enzymes, erythrocyte membrane structure, oxidative stress.

Oxidative stress caused by abrupt change in redox-condition of the body and activation of free-radical oxidation [8] plays an important role in molecular mechanisms of opium addiction. In turn, damaging effects of the oxidative stress contribute to dysmorphology in biomembranes; that intensifies the imbalance in harmonic-mediator exchange and causes the development of concurrent somatic pathologies in drug addiction [9, 13]. Recent researches pay much attention to different methods of energy-information influence (SCENAR-therapy, laser therapy, acupuncture, etc). It was found out that these methods are often more effective than pharmacological correction of metabolism imbalance [3, 12]. Today it is known that complex therapy for opium addiction at a stage of abstinence relief and next treatment stages, as well as arresting endogenous intoxication syndrome and correcting the harmonic-mediator status [2], should have evident anti-oxidant effect to limit damaging effects of the oxidative stress [8,9]. Our previous experimental and clinical researches show effectiveness of SCENAR-therapy as non-drug therapy for controlling free-radical

oxidation [7, 12]. So, there are certain pathogenetical reasons to include SCENAR-therapy into the complex of therapies for treating opium-addicted patients, it can also help to reduce total "pharmacological load" thus reducing the exertion of body detoxication systems.

The research was aimed to determine the influence of complex treatment including SCENAR-therapy on the indices of oxidant stress in the blood and structural state of erythrocyte membranes in opium addiction during abstinence relief.

## MATERIALS AND METHODS

56 opium-addicted patients (17-35 years old, disease duration - 1,5-10 years) participated in the clinicolaboratory examination. The patients got to clinic diagnosed with persistent abstinent syndrome and were divided into two groups depending on the therapy used:

1 group – patients undergone conventional treatment

2 group – patients undergone conventional treatment complemented with SCENAR-therapy.

SCENAR-97.4 was the device used in the research. All the sessions were done in the individual dose mode, 30-40 min each session, 10-14 days whole treatment course. Dynamic of body response was registered before, during and after the treatment course: we asked our patients to fill in the health status questionnaire, estimated redox condition of their body and indices of membrane homeostasis. The control group consisted of 15 almost healthy donors of appropriate sex and age.

Test material was venous blood stabilized by heparin 50 u/ml. Blood samples were centrifuged at 3000 r/min for 15 minutes and plasma was separated. Erythrocyte sediment was washed three times by 10ml of 0,15M NaCl solution in tris-HCl buffer pH 7,4 and then erythrocyte suspension with equal protein content (0,5mg/l) and 1% hemolysate was made. The intensity of free-radical processes was estimated according to H<sub>2</sub>O<sub>2</sub>-luminol-induced chemiluminescence parameters [14] and content of lipid peroxidation products. The content of primary lipid peroxidation products – diene conjugates - was determined according to the method [10], the content of secondary products – malondialdehyde – according to the method [11], end products – schiff bases – according to the method [15]. The state of antioxidant system was estimated by the activity of antioxidant ferments – superoxide scavengers [16] and catalases [4] in erythrocytes. Erythrocyte membrane stability was estimated according to the ectoglobular heumoglobin level of total peroxidase activity in blood plasma [6]. Structural condition of erythrocyte membranes was investigated using the method of piren fluorescent probe lateral diffusion [1]. Microviscosity of lipid bilayer and zones of protein-lipid contacts was determined by the value of piren excimerisation coefficients -  $F_3/F_M(334)$  and  $F_3/F_M(282)$ , that equal the correlation of fluorescence of its excimeric ( $F_e$ ) and monomeric ( $F_m$ ) forms at the exciting light wave length 334nm and 282nm. Restructure of membrane proteins was determined by the effectiveness of non-radiating energy transmit from membrane proteins to piren -  $F_0-F/F_0$ . Statistical analysis of the results was done using Student's t-criterion.

## RESEARCH RESULTS AND DISCUSSION

The research shows that in both clinical groups of opium-addicted patients in abstinence condition significant increase in effectiveness of free-radical processes (Table 1,2) in blood plasma and erythrocyte membranes is observed.

Table 1.

Intensity of H<sub>2</sub>O<sub>2</sub>-luminol-induced chemiluminescence and lipid peroxidation in blood plasma of opium-addicted patients in the multitherapy during the period of abstinent relief (M±m).

Indices	n	Treatment period	Chemiluminescence indices		Lipid peroxidation indices		
			H mm	Sm*10 <sup>4</sup> rel.unit	Diene conjugate nmol/ml	Malonic dialdehyde nmol/ml	Schiff bases u.fl/ml
Donors	10-15		40.0±7.0	74.0±13.0	12.38±1.71	20.87±1.58	1.062±0.009
1 group (conventional treatment)	15-22	Before	69.5±5.0*	108.0±7.0*	18.24±2.28*	38.98±8.76*	2.053±0.051*
		After	92.0±3.0**	119.0±4.0*	20.99±1.54*	43.49±11.16*	2.149±0.060*
2 group (conventional treatment + SCENAR-therapy)	15-20	Before	64.7±6.8*	129.4±8.0*	22.14±2.12*	37.63±2.87*	1.818±0.107*
		After	34.0±8.0**	74.9±8.5**	15.83±2.02*	28.61±8.18**	0.920±0.134**

Note: here and in Table 2,3

\* - reliability of differences compared to the checkout (donors), p<0.05-0.001;

\*\* - reliability of differences between indices before and after the treatment p<0.05-0.001

Table 2

Intensity of lipid peroxidation, activity of superoxide scavengers and catalase in erythrocytes of opium-addicted patients in the multitherapy during the period of abstinent relief (M±m).

Indices	n	Treatment period	Diene conjugate nmol/ml	Malonic dialdehyde nmol/ml	Schiff bases u.fl/ml	Superoxide scavengers u/mg hb	Catalase nmol H <sub>2</sub> O <sub>2</sub> /mg Hb
Donors	10-15		4.34±0.13	3.93±0.31	0.553±0.040	3.55±0.29	27.61±2.78
1 group (conventional management)	15-22	Before	7.02±0.31*	8.80±0.32*	0.765±0.068*	2.66±0.13*	19.88±2.12*
		After	8.19±0.41**	10.03±1.0*	0.543±0.059**	3.15±0.36*	19.16±0.63*
2 group (conventional management + SCENAR-therapy)	15-20	Before	9.63±1.41*	8.33±0.34*	0.997±0.085*	2.57±0.15*	17.74±2.06*
		After	5.47±0.53*	5.57±0.24**	0.459±0.042**	3.37±0.12**	24.10±2.13*

The indices of H<sub>2</sub>O<sub>2</sub>-luminal-induced chemiluminescence – quick flash height (m) and light sum – 49-79% exceeds the norm (donors) before treatment, that signs extra generation of oxygen active forms, which have high cytotoxic potential and can initiate lipid peroxidation. Table 1 and 2 show that the content of molecular products – diene conjugate, malonic dialdehyde, schiff bases - in blood plasma and erythrocyte membranes in both groups of patients exceeds the control level by 38-122%. During the increase of free-radical process intensity in erythrocytes of addicted patients we can observe inhibition of antioxidant ferments of superoxide scavengers and catalase by 25-36%, which control the free-radical process at the stage of oxygen activation and origin of lipid peroxidation chain process. So, we can observe the development of oxidative stress in opium-addicted patients and that has a great number of damaging effects. That can be proved both by our research results [8,9] and by researches made by other authors [13].

Our research showed that in the 1 group of addicted patients that undergone conventional treatment intensity of free-radical processes in the blood remains as high, as it was registered before the treatment. The only exception is that after the treatment the content of lipid peroxidation end products – schiff bases - in erythrocyte membranes decreased by 29% (Table 1,2).

In the 2 group of patients that undergone conventional treatment combined with SCENAR-therapy, intensity of free-radical processes in blood, in a whole, returns to the stationary level typical for the norm. During the treatment chemiluminescence parameters decrease by 42-47% and the content of lipid peroxidation products in blood plasma reduces by 24-49%, in erythrocyte membranes – 35-54% relative to the initial level, except malonic dialdehyde content, which remains 42% higher than the control index even after the treatment (Table 1,2).

In the 1 group of patients, where the treatment didn't influence the intensity of free-radical processes in the blood, we can observe the disfunction of conjugated antioxidant enzymes in erythrocytes. Superoxide dismutase activity during the treatment approaches the control indices, while catalase activity is by 31% inhibited compared to the norm. On a contrary, in the 2 patient group, where the treatment helped to normalize most of the chemiluminescence and lipid peroxidation parameters, during the treatment we can observe stimulation of activity of superoxide dismutase and catalase in erythrocytes. Activity increment of antioxidant enzymes in erythrocytes is 31-96% compared to the initial background (Table 2).

So, complementing complex treatment of opium-addicted patients with SCENAR-therapy significantly decreases the manifestation of oxidative stress by activating the most important erythrocyte enzymes and decreasing the intensity of free-radical processes in the blood. So, restoration of redox state in the blood can be considered as one of the most important mechanisms of therapeutic impact of SCENAR-therapy in treating drug addiction.

Uncompensated activation of lipid peroxidation in erythrocyte membranes among patients of the 1 and 2 groups before the treatment causes destabilization and destructuring. Significant level increase of ectoglobular hemoglobin of total peroxidase activity (66-88% from the norm) in blood plasma in both patient groups before the treatment (Table 3) shows that levels of ectoglobular hemoglobin and total peroxidase activity in blood plasma are considered to be sensitive parameters of erythrocyte membrane stability [6], and their increase reflects the increase of plasma prooxidant

potential as it contributes to additional activation of lipid peroxidation due to formation of oxygen active forms and hemoglobin ferril– radical.

Table 3 shows that erythrocyte membranes of drug-addicted patients from two groups observe the 17-21% decrease of piren probe excimerization coefficient  $F_e/F_M(334)$  and the 27-33% increase of the  $F_e/F_M$  parameter (282). It's known that degree of piren excimerization is in the inverse dependence on lipid phase microviscosity [1, 5]. The research results show opposite changes in microviscosity of different erythrocyte membrane compartments, that increases in lipidic bilayer and decreases in protein–lipid contact zones presented by annular lipids that form microenvironment of membrane proteins.

Table 3

Ectoglobular hemoglobin level, total peroxidase activity in blood plasma and structural state of erythrocyte membranes among opium-addicted in the multitherapy during the abstinent relief ( $M \pm m$ ).

Indices	n	Treatment period	Blood plasma		Erythrocyte membranes		
			Ectoglobular hemoglobin in mcM/l	Total peroxidase activity un/ml	$F_e/F_M(334)$	$F_e/F_M(282)$	$F_0-F/F_0$
Donors	10-15		4.84±0.44	3.56±0.45	0.75±0.05	0.98±0.02	0.152±0.006
1 group (conventional treatment)	15-22	before	8.94±0.42*	5.91±1.30*	0.59±0.03*	1.24±0.07*	0.129±0.004*
		after	9.38±2.46*	6.51±1.69*	0.62±0.04*	1.11±0.10*	0.135±0.003*
2 group (conventional treatment + SCENAR-therapy)	15-20	before	8.51±0.75*	6.70±1.30*	0.62±0.03*	1.30±0.08*	0.128±0.006*
		after	4.98±0.60*	3.05±0.26**	0.80±0.05*	1.01±0.11*	0.144±0.006

At the same time it was registered that  $F_0-F/F_0$  parameter decreased by 15-16% and that indicates the decrease in effectiveness of nonradiating energy transfer of electronic excitation from membrane proteins to piren and indicates destructuring in membrane proteins of erythrocytes. Modification of erythrocyte membrane structure inevitably causes inhibition in functionally important membrane processes, as well as impairment of membrane viscoelastic properties and decrease in hemolytic resistance of erythrocytes.

After the treatment course in the 1 group of patients most of the structure disturbances in the erythrocyte membrane remain the same, except the normalization of annular lipid microviscosity  $F_e/F_M(282)$  (Table 3).

Conventional treatment combined with SCENAR-therapy in the 2 group of patients contributes to normalization of erythrocyte membrane structural parameters; fluidity of lipid bilayer and annular lipids normalizes, structural changes in membrane proteins eliminate and that improves the structure and function of erythrocyte membranes. It seems that there are different ways to produce membrane stabilizing effect of the SCENAR-therapy: a) normalize the structure of erythrocytes circulating in blood channel by inhibiting free-radical processes and stimulating antioxidant enzymes; b) change the structure of erythrocyte population to its rejuvenation. It's known that

young erythrocytes are characterized by hyperplasticity and optimal viscoelastic properties [5].

So, complementing complex treatment of opium-addicted patients with SCENAR-therapy helps to decrease negative manifestations of oxidative stress by decreasing the intensity of free-radical processes, stimulating the antioxidant protection enzymes in blood, increasing stability and restoring structural homeostasis of the erythrocyte membranes.

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