SCENAR-therapy influence on the neurotransmitter systems state and oxidative stress indexes at opium addiction

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The chronic entry of opium products in the organism constitutes the element starting the following cascade of pathological reactions which affect the key parts of the metabolism that results in the development of psychic and physical dependence. The chronic dysfunction of dopamine (DA) neurotransmitter system of the brain affecting the reinforcement system [1, 2] constitutes the neurochemical basis of the dependence on the opiates phenomenon and other psychoactive substances (PAS). According to PAS, the clinical aspects act as hypercomplex mosaic phenotype (addiction phenotype) determined by multiversion interaction of the gene system regulating the dopamine mediation [3, 4].

The deep shifts in the mediator exchange, microsomal oxidation involvement in the opiates biotransformation, periodic hypoxia at opium addiction [1, 5, 6] allow to suppose the free-radical processes (FRP) and oxidative stress playing a key role in this disease pathogenesis.

The oxidative stress is defined as the state of the dynamic equilibrium shift in the prooxidants-antioxidants system in favor of the reinforcement of free radical oxidation against the background of the intensity and imbalance of antioxidant system components [7]. It was demonstrated that the progressively intensifying disturbances of structural-metabolic homeostasis are observed at addictive disorders and manifested in the reinforcement of free radical oxidation (FRO), disorder of biomembranes structural condition interrelated with the dysfunction of the hormonal-mediator exchange [1, 7, 8, 9]. All these pathological changes are accompanied with somatic disorders and form a molecular basis for the addiction being targets of the metabolism correction at narcomania therapy. However, the majority of narcomania treatment methods are drug treatment methods [10] that increase the pharmacological and, particularly, «neuroleptic» load and the intensity of body detoxification systems. Thereby, the development of effective drug-free methods of narcomania treatment directed to normalize the metabolism key links disordered at this pathology is of special interest. The medical technology like SCENAR-therapy meets these requirements. SCENAR-therapy provides correction and normalizing effect on the metabolism by stimulating the endogenous reserves [11, 12]. We have

demonstrated the high effectiveness of SCENAR- therapy as drug-free method applied to arrest the oxidation process [11, 13] in the experimental and clinical researches performed before.

Accordingly, the research objective was to determine the effect provided by the combination treatment including SCENAR-therapy on the dopamine- and serotonergic neuromediation and prooxidant-antioxidant status in the blood of the patients suffering from opium addiction.

Materials and methods

The clinical-laboratory examination has been applied to 120 patients with diagnosis "dependence on the opiates" (F-11 MKB-10) being treated in the department No.2 of State Health Care Facilities "Psychoneurologic Dispensary" (Rostov-on-Don) within the period of 2003-2007 yrs.. All the patients were men, their average age - 24,5 years old, the period of regular opium group drugs intake - from 1 up to 6 years. Two groups of patients were examined: 1st group – patients who had received combination treatment according to the generally accepted scheme; 2nd group – patients who had received generally accepted treatment combined with SCENAR-therapy. The patients of each clinical group were examined in the abstinency state before be treated, after the treatment had been completed (10 days later after they had been admitted to the hospital) and during the remission (4-6 months after the drug had been discontinued). SCENAR-therapy was applied in the individually dosed mode using SCENAR-97,4 device with total action duration of 30-40 min within 10-14 days during the abstinency and remission. Before starting, during the process and also after the treatment course had been completed, the dynamics of psychological, physiological and biochemical reactions of the body was considered, the patients' well-being was determined using the questionnaire. The control group included 20 apparently healthy donors of the corresponding sex and age.

The test material: venous blood stabilized with heparine - 50 U/ml. The blood samples were centrifuged for 15 min at 3000 r/min, the plasma was separated and 1 % hemolysate was obtained.

The reversed-phase version of high-efficiency liquid chromatography was used to determine the dopamine and serotonin.

The lipid peroxidation intensity was evaluated based on the level of the malondialdehyde (MDA) secondary product determined according to the method [14], superoxide generating activity (SGA) – according to the method [15], velocity of hydrogen dioxide utilization [V_{H2O2}] – according to the method [16]. The state of the antioxidant system of the blood plasma was determined based on to the ceruleoplasmin activity [17] and α- tocopherol content (α-Tph) [18].

The activity of the erythrocytes antioxidant system was defined based on the activity of superoxide dismutase (SOD) [15], catalase [16], glutathione peroxidase [19] and reduced

glutathione level [18]. The statistical processing of the results was performed using Student t-test [20].

Research Results and Discussion

The performed research proves that the dopamine level increases by 4,7-6,2 and the serotonin level increases by 1,5-1,8 in the blood of the patients included in both clinical groups and suffering from the opium addiction as compared with the control value (Table 1). We can suggest that the increase of DA and serotonin levels in the patients' blood plasma is connected with the "rewarding" system neurons activation by the opiates from which the catecholamine – and serotonergic neurons receive the amplified signal to discharge the mediators from the depot in the blood [1, 2]. At that, the inhibition of the monoaminooxidase A and other ferments participating in DA and serotonin metabolism, and also suppression of specific transporters providing reuptake of the neuromediators [1, 2] are observed in the blood of the patients suffering from narcomania.

The effectiveness of the traditional therapy and combination treatment applying SCENAR-therapy was compared by evaluating the neuromediation system state in the course of research. After the treatment completed, the DA level considerably decreased in the blood plasma of the 1st group patients who had received traditional treatment but it was kept for 71 % and 111 % above the norm value, the DA level was close to the norm value (Table 1) during the remission. The serotonin content in the blood plasma of the patients included in the 1st group was normalized after the abstinency had been removed, and it exceeded the norm value for 61% during the remission. In the 2nd group of patients who had received the treatment combined with SCENAR-therapy, the normalization of DA and serotonin level was observed both after the abstinency had been removed and during the remission (Table 1).

So, SCENAR-therapy applied as a part of the combination treatment to the patients suffering from opium addiction, in general, provides normalization of the activity of dopaminergic and serotonergic mediator systems that decreases the addiction level and provides stable remission. We can suggest that SCENAR-therapy removes the neuromediator systems hyperactivation by various means which include activating effect on the ferments of dopamine and serotonin biotransformation and also optimization of the structure and function of the receptor apparatus and neuromediators transporters, due to the unique physical specifications (not damaging bioregulated high-amplitude electrical pulses, pulsed field which gives rise to high-frequency vibrations of the tissues).

It is not improbable that the normalizing action of SCENAR-therapy is provided by antioxidant action which we have demonstrated before at various pathologies [11, 13]. It is

known that FRO inhibition may provide functional restoration of the ferments, receptors, membrane carriers [7].

Accordingly, we investigated the influence provided by SCENAR-therapy on the oxidative stress indexes at opium addiction. The increase of superoxide generating activity (SGA) of the blood plasma for 125-129 % is observed at abstinency in the clinical groups of patients, while the hydroperoxide ($\upsilon_{н2о2}$) utilization velocity in the plasma decreased for 41-52% relative to the control value (Table 2). It may result in considerable increase of the hydroperoxide level and additional production of active oxygen forms (AOF). The periodic respiratory and tissue hypoxia [5, 6] should be noted among various causes of FRP intensification at opium addiction, and also hypercatecholaminemia mentioned in this work. The steep rise of the dopamine level in the blood at narcomania during the abstinency [21] results in its autoxidation in the course of which the superoxide anion radical appears [22].

The excessive formation of AOF at abstinency starts the lipid peroxidation process in the blood of the patients included in both clinical groups (Table 2, 3) which is approved by the MDA level increase in the plasma and erythrocytes for 80-92% relative to the norm value. The lipid peroxidation intensification at narcomania may result in oxidative damage of the biomolecules and cellular structures.

The important role in FRP regulation belongs to the antioxidant ferments SOD, catalase, GPO acting in couple and inhibiting the lipid peroxidation at the stage of oxygen activation, initiation and branching of the chain process. The inhibition of SOD and catalase for 25-36 %, GPO – for 28-30 %, GSH level decrease – for 17-22 % (Table 3) are observed in the erythrocytes of the patients with abstinence symptoms included in both groups.

The important components of the antioxidant system of the blood plasma include copper polyfunctional protein ceruloplasmin (CP) and low-molecular antioxidant α- tocopherol (α-Tph).

At abstinency, the oxidase CP activity in the blood plasma of the patients of both groups increased for 63-115 % as compared with the norm value (Table 2) that may be considered as compensatory reaction due to ferroxidase and SOD-like activity of CP. The decrease of α-Tph level for 41-43 % is observed in the blood plasma of the patients included in both groups before be treated. α-Tph is an effective quencher of singlet oxygen and restores the lipoperoxyl radicals [7].

So, the following is observed at opium addiction during the abstinency: the free radical homeostasis disorder and shift of prooxidant-antioxidant equilibrium in the blood in favor of FRO intensification that results in the oxidative stress development with multiple damaging action.

The comparative examination of two groups of patients suffering from narcomania who received different treatment (traditional treatment and traditional treatment combined with

SCENAR-therapy) demonstrates more expressed curative effect of the bioregulated electric pulse therapy. If applying the traditional treatment to the patients of the 1st group, the dynamics of the most investigated biochemical indexes proves that the oxidative stress state is kept. So, after the treatment course was completed and during the remission, the SGA level and MDA content in the plasma and erythrocytes considerably exceeds the norm, and υ_{H2o2} is kept below the control value in the plasma (Table 2, 3). It appears that the noncompensated activation of FRP in the blood of the patients included in the 1st group after the treatment completed is connected with partial inhibition and reduction of the antioxidant blood system capacity. After the treatment course was completed and also during the abstinency, the SOD and catalase activities are kept decreased in the erythrocytes of the 1st group patients, but they will be normalized later during the remission (Table 3). The GPO activity and GSH level had decreased before the treatment started, now they are kept below the norm value after the therapy course was completed and during the remission. However, after the abstinency was removed, the CP oxidase activity decreases but α-Tph level is kept below the norm value in the blood plasma as after the treatment was completed and during the remission (Table 2).

In the second group of patients who had traditional treatment combined with SCENAR-therapy, FRP intensity decreases in the blood in general. But the complete normalization of SGA levels in the blood plasma and this of MDA in the erytrocytes is not observed against the background of the positive dynamics of the oxidation-reduction state indexes in the second group of patients. Application of SCENAR-therapy as a part of combination treatment of the patients included in the second group results in stimulation of antioxidant blood system. After the abstinency was removed and during the remission, SCENAR-therapy provides increase of the activity of SOD, catalase, GPO and GSH level (Table 3). At the same time, the hydrogen peroxide (υ_{H2o2}) utilization velocity is normalized in the blood plasma and the oxidase CP activity is close to the α-Tph content norm within the same treatment period.

So, SCENAR-therapy application as a part of combination treatment of the patients suffering from the opium addiction considerably decreases the manifestation of the oxidative stress both at abstinency removal and during the remission by stimulating the important components of antioxidant blood system. We can conclude that SCENAR-therapy is a high-effective, safe drug-free method of activation and strong stabilization of compensatory-adaptive reactions directed to correct and restore the structural-metabolic and neuro-mediator homeostasis in patients suffering from opium addiction.

Literature

1. Анохина И.П., Веретинская А.Г., Васильева Г.Н. и др. О единстве биологических механизмов индивидуальной предрасположенности к злоупотреблению различными психоактивными веществами // Физиология человека, 2000, Т. 26, № 6, с. 76-83.

2. Nestler E. Molecular mechanisms of drug addiction // J. Neuroscience, 1992, Vol. 12, № 7, p. 2439-2450.

3. Анохина И.П., Иванец Н.Н., Шамакина И.Ю. и др. Современные проблемы генетики зависимости от психоактивных веществ // Наркология, 2004, № 6, с. 76-83.

4. Кибитов А.О., Воскобоева Е.Д., Бродянский и др. Полиморфизм гена транспортера дофамина (ДАТ1) у больных алкоголизмом и героиновой наркоманией с отягощенной наследственностью // Вопр. наркологии, 2009, № 3, с. 78-89.

5. Фридман Л.С., Флеминг Н.Ф., Робертс Д.Х. и др. Наркология / Под ред. С.Е. Хайман. – М.: СПб: «Изд-во БИНОМ» - «Невский Диалект», 1998. – 318 с.

6. Шабанов П.Д. Руководство по наркологии. – СПб: Изд-во «Лань», 1999. – 352 с.

7. Меньщикова Е.Б., Зенков Н.К., Ланкин В.З. и др. Окислительный стресс: Прооксиданты и антиоксиданты. – М.: Фирма «Слово», 2006. – 556 с.

8. Усманова Н.Н. Соматические осложнения опийной наркомании в подростковом возрасте и роль свободнорадикальных процессов в их патогенезе. Автореф. дисс… к.м.н. – М., 2000, 20 с.

9. Овсянников М.В., Масловский С.Л., Милютина Н.П. Структурное состояние мембран эритроцитов в патогенезе опийной наркомании // Биол. мембраны, 2005, Т. 22, № 2, с. 100-104.

10. Рохлина М.Л., Козлов А.А., Мохначев С.О. и др. Принципы фармакотерапии опийной наркомании // Наркология, 2002, № 11, С. 28-30.

11. Тараканов А.В., Гринберг Я.З., Милютина Н.П. Универсальные механизмы действия СКЭНАР при окислительном стрессе // Рефлексотерапия, 2003, № 4(7), С. 41-45.

12. Гринберг Я.З. СКЭНАР-терапия и СКЭНАР-экспертиза. Некоторые аспекты // Рефлексология. Научно-практ.ж., 2005, № 3 (7), С. 5-10.

13. Овсянников М.В., Чирко В.В., Морозов С.Г., Милютина Н.П. Аддиктивные расстройства у больных шизофренией. М.: Изд-во Книжкин Дом, 2008. – 256 с.

14. Стальная И.Д., Гаришвили Т.Д. Метод определения малонового диальдегида с помощью тиобарбитуровой кислоты // Современные методы в биохимии. – М.: Медицина, 1977, с. 66-68.

15. Fried R. Enzymatic and non- enzymatic assay of superoxide dismutase // Biochem., 1975, V. 57, N 5, p. 657-660.

16. Королюк М.А., Иванова Л.И., Майорова И.Г. и др. Метод определения активности каталазы // Лаб. дело. – 1988, № 1, с. 16-19.

17. Колб В.Г., Камышников В.С. Справочник по клинической химии. – Минск: Беларусь, 1982, с. 289-292.

18. Арутюнян А.В., Дубинина Е.Е., Зыбина Н.Н. Методы оценки свободнорадикального окисления и антиоксидантной системы организма. Методические рекомендации. – СПб.: ИКФ «Фолиант», 2000, с. 93-94.

19. Моин В.М. Простой и специфический метод определения активности глутатионпероксидазы в эритроцитах // Лаб. дело, 1986, № 12, с. 724-727.

20. Лакин Г.Ф. Биометрия. – М.: Высшая школа, 1980. – 293 с.

21. Анохина И.П., Иванец Н.Н., Борисова Е.Д., Дробышева В.Я. Итоги научно-исследовательских работ за 2008 г. по комплексной проблеме наркология, координируемой научным советом РАМН // Вопр. наркологии, 2008, № 4, с. 3-13.

22. Болдырев А.А. Двойственная роль свободнорадикальных форм кислорода в ишемическом мозге (обзор)//Нейрохимия,1995, Т.12, № 3, с.3-13.

Table 1

Influence provided when applying various methods of the combination treatment on the dopamine and serotonin content in the blood plasma of the patients suffering from the opium addiction, M±m, n = 15-35

Indexes, Unit of Measurement	**Donors**	**Traditional Treatment**			**Traditional Treatment + SCENAR-therapy**		
		1st group			**2nd group**		
		Abstinency	**After the treatment completed**	**Remission**	**Abstinency**	**After the treatment completed**	**Remission**
Dopamine (DA) ng/l	205,0 ± 30,0	1271,0± 12,0*	351,0 ± 54,0*	220,0 ± 20,0**	967,0 ± 161,0*	207,0 ± 15,0**	223,0 ± 6,7**
Serotonin (S) ng/l	11693 ± 214	21263 ± 174*	14503 ± 111**	18832 ± 1864**	17568 ± 1536*	9636 ± 749**	11451 ± 669**

Note: * – Reliability of differences as compared with the donors

** - Reliability of differences relative to the level before the treatment started

Table 2

State of the components of prooxidant and antioxidant system of the blood plasma of the patients suffering from opium addiction when applying various treatment methods, M±m, n = 20-30

Indexes, Unit of Measurement	**Donors**	**Traditional Treatment**			**Traditional Treatment + SCENAR-therapy**		
		1st group			**2nd group**		
		Abstinency	**After the treatment completed**	**Remission**	**Abstinency**	**After the treatment completed**	**Remission**
SGA U/ml	-11,56 ± 3,27	2,63 ± 0,28*	1,92 ± 0,46***	0,93 ± 0,18***	2,91 ± 0,37*	1,53 ± 0,42***	0,75 ± 0,20***
υ_{H2O2} nml H_2O_2/ml/min	9,73 ± 0,55	5,74 ± 0,92*	5,84 ± 1,46*	7,55 ± 0,67*	4,66 ± 0,54*	10,44 ± 1,87**	8,02 ± 1,47**
CP mcM/l	1,23 ± 0,05	2,01 ± 0,24*	0,70 ± 0,07***	0,84 ± 0,22 **	2,65 ± 0,04*	1,27 ± 0,24**	1,14 ± 0,15**
α-Tph mcM / l	35,11 ± 4,08	20,79 ± 4,33*	27,39 ± 0,54*	25,41 ± 1,28*	19,86 ± 3,25*	30,68 ± 3,13**	29,87±2,34***
MDA nmole/ml	20,87 ± 1,58	39,98 ± 8,76*	43,49 ± 11,16*	29,44 ± 2,11***	37,63 ± 2,87*	28,61 ± 8,18**	18,93 ± 1,05**

Note: * – Reliability of differences as compared with the donors

** - Reliability of differences relative to the level before the treatment started

Table 3

Lipid peroxidation intensity and antioxidant ferments activity in the erythrocytes of the patients suffering from the opium addiction when applying various treatment methods, M±m, n = 20-30

Indexes, Unit of Measurement	**Donors**	**Traditional Treatment**			**Traditional Treatment + SCENAR-therapy**		
		1st group			**2nd group**		
		Abstinency	**After the treatment completed**	**Remission**	**Abstinency**	**After the treatment completed**	**Remission**
MDA nmole/g Hb	3,93 ± 0,31	8,8 ± 0,32*	10,03 ± 1,0*	8,34 ± 0,35*	8,33 ± 0,34*	5,57 ± 0,24***	5,83 ± 0,35***
SOD units/ml	3,55 ± 0,29	2,66 ± 0,13*	3,15 ± 0,36*	3,64 ± 0,23	2,57 ± 0,15*	3,37 ± 0,12**	4,43 ± 0,30***
catalase nmole H_2O_2/mg Hb/min	27,61 ± 2,78	19,88 ± 2,12	19,16 ± 0,63*	25,19 ± 2,87**	17,74 ± 2,06*	24,10 ± 2,13**	23,68 ± 1,29**
glutathione peroxidase (GPO) mcM/min/g Hb	256,9 ± 12,8	180,9 ± 17,8*	176,2 ± 9,6*	197,0 ± 6,0*	183,9 ± 7,8*	264,8 ± 13,9**	218,2± 8,6**
glutathione (GSH) mcM/mg Hb	24,7 ± 0,7	19,3 ± 1,3*	17,9 ± 0,8*	20,14 ± 1,4*	20,6 ± 0,9*	22,4 ± 1,0	23,1 ± 3,4

Note: * – Reliability of differences as compared with the donors

** - Reliability of differences relative to the level before the treatment started