CLINICAL STUDY

Prooxidative/antioxidative homeostasis in heroin addiction and detoxification

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Abstract

Background: **Long-term heroin abuse is related to pathological changes in many organs mediated by oxidative stress (OS).**
Objectives: **Estimation of systemic OS and antioxidant capacity in heroin addiction and detoxification provides information about prooxidant/antioxidant homeostasis in heroin misuse and need for antioxidant supplementation.**
Methods: **OS was evaluated by the measurement of plasma reactive oxygen metabolites using spectrophotometric method and plasma lipid peroxidation by its end product – malondyaldehyd using Tiobarbituric Acid Reactions Substances method. The extracellular antioxidant capacity was estimated using OXY-adsorbent test.**
Results: **This cross-sectional study includes 68 patients: 46 heroin addicts (20 patients on chronic heroin abuse, 19 patients on conventional method of detoxification and 7 patients on opioid antagonist – naltrexone) and 22 patients as a control }group. Increased OS was found in the heroin group (d-ROMs 349.3±102.2 UCarr, MDA 4.0±0.4 µmol/L) compared to the group on detoxification (d-ROMs 230.2±96.4 UCarr; MDA 3.6±0.3 µmol/L) and control group (d-ROMs 264.1±30.9 UCarr; MDA 3.7±0.2 µmol/L). TAC was decreased in the heroin group (324.5±75.0 µmol HClO/ml) and restored during conventional detoxification (371.8±25.1 µmol HClO/ml), but not completely in the group with naltrexone treatment (335.6±16.9 µmol HClO/ml) compared with controls (395.4±35.6 µmol HClO/ml).**
Conclusion: **Long-term heroin abuse stimulates a progressive systemic oxidative stress which increases the extracellular antioxidants consumption and develops conditions for chronic heroin toxicity *(Fig. 1, Tab. 4, Ref. 35).* Full Text (Free, PDF) *www.bmj.sk.***
Key words: heroin addiction, oxidative stress, free radicals, lipid peroxidation, total antioxidant capacity.

Abbreviations: OS – oxidative stress, ROS – reactive oxygen substances, MDA – malondyaldehyde, BMI – body mass index, d-ROMs – reactive oxygen metabolites, TBA – tiobarbituric acid, TAC – total antioxidant capacity

Heroin abuse is a worldwide health problem that provoked many investigations to study its mechanism of action. Lately, the mode of heroin-induced toxicity was tried to be explained at the molecular level considering electron transfer processes, free radical production and oxidative stress (OS). Focal glomerulosclerosis (1), hepatotoxicity with a reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase levels (2), histamine liberation from mast cells (3), neurotoxicity with spongiform leucoencephalopathy (4), neuroteratogenicity (5) and oxidative damage of DNA (6) are some of the pathological features in chronic heroin toxicity generated by local rearrangement of antioxidant homeostasis. Recently, the development of a systemic OS was noted as a result of opioid-stimulated free radicals production. An increased lipid peroxidation was found in vitro and in vivo in heroin consumption (7, 8). Decreased levels of antioxidants and antioxidases are referred in states of chronic heroin (morphine) abuse (9, 10).

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The OS, the state of an increased production of free radicals and reactive oxygen substances (ROS) and decreased antioxidant capacity, leads to the oxidation of polyunsaturated fatty acids in lipids, thiols in proteins and nucleic basis in DNA. These processes induce disrupting in cellular signaling, apoptosis and necrosis (diseases or death) (11). Measurement of the levels of free radical or ROS production, metabolites of lipid peroxidation such as malondyaldehyde (MDA) and antioxidant capacity may give information about an antioxidant homeostasis under heroin stimulation and heroin-induced toxicity.

The aim of this study is to evaluate the prooxidant/antioxidant homeostasis in addicts using heroin and addicts undergoing detoxification.

Materials and methods

Materials

A cross sectional study was designed for 46 heroin addicts and 22 healthy controls. The addicts were divided in the 3 following groups: I. Addicts who consume impure, "street" heroin by intravenous or intranasal application, II. Addicts included in the conventional detoxification program from the 8th to 10th day of detoxification (chlorpromazine 100 mg three times daily per os, diazepam 10 mg three times daily per os), III. Addicts treated with opioid agonist naltrexone 50 mg a day (per os). The patients from all groups are active cigarette smokers. Control group data were from the basis for antioxidant homeostasis at the Institute for Physiology and Anthropology, Medical Faculty, Skopje.

The patients and controls (68) were young people under 30 years, 7 female and 55 male, with BMI in referent range (Tab. 1).

To test the homogeneity of the groups, the results were statistically checked comparing the groups in age, sex, BMI and the route of drug administration and duration of heroin abuse. The difference in age ($F=1{,}098$, $p>0.05$), sex ($chí^2=2.57$, $df=2$, $p>0.05$) and BMI ($F=0.086$, $p>0.05$) between the groups was statistically not significant with a statistically significant domination of males in the each group ($chí^2=33.882$, $df=1$, $p<0.01$). The patients in heroin, detoxification and naltrexone group showed a long period of heroin use (average 7.20±3.13 years) and no statistical difference in duration of heroin use between groups ($F=0.362$, $p>0.05$) was found. More addicts have used heroin intravenously compared to inhalation ($chí^2=28.174$, $DF=1$, $p<0.01$) but no statistically significant difference was found between the groups considering the route of heroin administration ($chí^2=0.102$, $df=2$, $p>0.05$) (Tab. 1).

Tab. 1. Group distribution of patients number, age, sex, BMI, route of drug administration and duration of addiction.

Group	Pts (n)	Age (years)	Sex (m/f)	BMI (kg/m^2)	Adminst. (Inh/i.v.)	Addiction duration (years)
1	20	24.6±5.5	17/3	22.82±6.1	2/18	7.63±3.46
2	19	25.2±5.3	18/1	22.59±6.0	2/17	6.84±3.0
3	7	21.8±3.1	5/2	22.58±5.9	1/6	6.85±2.67
4	22	23.6±4.2	18/4	23.45±6.9	–	–
Sign.	–	p>0.05	p>0.05	p>0.05	p>.0.05	p>0.05

Pts – patients, n – number of patients, m/f – male/female, BMI – body mass index, Administ – route of drug administration, INH – inhalation, i.v. – intra venous use, Sign – significance

Addiction is defined as a repeated, urged seeking or useing substances despite the undesirable social, psychological and/or physical consequences (psychological and physical addiction and tolerance). This condition includes also new detoxified heroin addicts who don't suffer from abstinence syndrome or tolerance but anyway seek for opioids and would relapse in an active misuse of heroin in the absence of further treatment in the National Institute of Drug Abuse (12).

Including criteria were following

Regular heroin consumption for at least 1 year,

Age under 30 years

Criteria for not including the patients were following:

HIV seropositivity,

Acute and chronic diseases (with the exception of the asymptomatic carriers of hepatitis B and C with normal range of aminotransferases),

Regular supplementation with antioxidants in the last 4 weeks,

Consumption of more than 2 glasses of alcohol per day,

Positive findings of psychostimulants' products in the urine (cocaine, cannabis sativa, MDMA),

Disturbances of nutritional status (BMI less than 18.5 kg/m^2 and more than 25 kg/m^2),

Regular use of medications which act as donors of nitric oxide (statines, ACE inhibitors, AT1 receptors antagonists, nitrites).

An exclusion criterion was a finding of psychostimulants' metabolites in urine during the detoxification.

Methods

Examination methods included:

1) History and physical examination,

Laboratory tests (serological testing for HIV, anti HBV antibodies and anti HBC antibodies using Microparticle Enzyme Immunoassay method),

Toxicological urine testing for metabolites of heroin, methadone, cannabis sativa, cocaine, MDMA and benzodiazepines, performed with fluorescence polarization immunoassay method at the Institute for forensic medicine).

2) Estimation of prooxidative and antioxidative parameters;

Venous blood was taken in the period from 10–12 p.m. and collected in heparinized tubes. MDA was determined in serum, TAS and d-ROMs were measured in plasma. Analyses were performed at the Institute of Physiology and Anthropology, Medical Faculty, Skopje.

a) D-ROMs (reactive oxygen substances) were determined by a colorimetric method using DIACRON instrument. Hydroperoxides react with a chromogenic substrate and develop a colored complex what is quantifiable by a spectrophotometric method

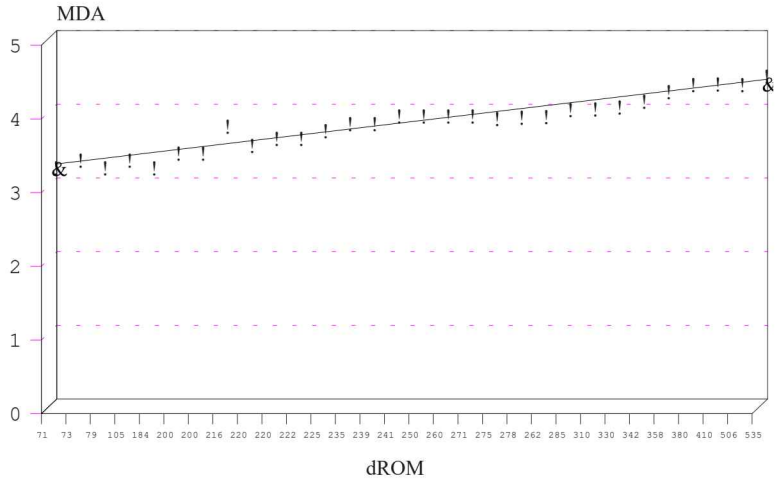

Fig. 1. Correlation between plasma levels of d-ROMs (U Carr) and MDA (µmol/l).

(wavelength 546 nm optical path 1 cm, temperature 37 °C). More than 300 U. Carr are pathological values (13).

b) The levels of the stable intermediary product of lipid peroxidation – malondyaldehyde (MDA) was determined using its reactivity with Tiobarbituric acid (TBA) which produces a specific absorption spectrum (Yagi et al, 1967). Supernatant is used for the measurement with spectrophotometer, Ex 515 nm, Em 550 nm. Referent range is 3.2–4.0 µmol/l.

c) The Total Antioxidant Capacity (TAC) was performed by the OXY adsorbent test (DIACRON). The un-neutralized radicals of HClO react with a chromogenic substrate forming a colored complex, which is photometrically measured with a maximum peak of absorbance at 546 nm, at room temperature. The concentration of the colored complex is indirectly proportional to the antioxidant capacity. Values less than 350 µmol HClO/ml indicate a lower extracellular antioxidant status.

Statistical data were processed using the SPSS 11 program. All data were analyzed using a descriptive statistics, chi^2-test, One-way ANOVA for numerical variables for testing the inter group differences and low summary difference (LSD) for defining the groups with a statistically significant difference. Correlations were tested using the Pearson – r for numerical data. Values $p<0.05$ and $p<0.01$ were considered statistically significant.

Results

Higher values of d-ROMs were found in the group on heroin compared to the group on conventional detoxification, naltrexone and control group (Tab. 2).

Statistical testing showed a significant inter-group difference (df=3, F=6.651, p=0.001) and defined the group with a statistical difference. Heroin group showed significantly higher levels

Tab. 2. Group distribution of d-ROMs' mean values and intergroup statistical difference.

Group	n	Mean values of dROMs±SD (U.Carr)	Group	Significance
			2	p<0.05
1	17	349.3±102.2	3	n.s.
			4	p<0.05
			1	p<0.05
2	17	230.2±96.4	3	p<0.05
			4	n.s.
			1	n.s
3	7	306.3±99.9	2	p<0.05
			4	n.s.
			1	p<0.05
4	22	264.1±30.9	2	n.s.
			3	n.s.

1 – heroin, 2 – conventional detoxification, 3 – naltrexone, 4 – controls, n – number of pts

Tab. 3. Distribution of MDA mean values and intergroup statistical differences.

Group	n	Mean values of MDA±SD (µmol/l)	Group	Significance
			2	p<0.05
1	17	4.0±0.4	3	n.s.
			4	p<0.05
			1	p<0.05
2	16	3.6±0.3	3	n.s
			4	n.s.
			1	n.s
3	7	3.8±0.4	2	n.s.
			4	n.s.
			1	p<0.05
4	22	3.7±0.2	2	n.s.
			3	n.s.

1 – heroin, 2 – conventional detoxification, 3 – naltrexone, 4 – controls, n – number of pts

Tab. 4. Group distribution of TAC mean values and intergroup statistical difference.

Group	n	Mean values of TAC±SD (μmol HClO/ml)	Group	Significance
			2	p<0.05
1	19	324.5±75.0	3	n.s.
			4	p<0.05
			1	p<0.05
2	16	371.8±25.1	3	n.s
			4	n.s.
			1	n.s
3	7	335.6±16.9	2	n.s.
			4	p<0.05
			1	p<0.05
4	22	395.4±35.6	2	n.s.
			3	p<0.05

1 – heroin, 2 – conventional detoxification, 3 – naltrexone, 4 – controls, n – number of pts

of d-ROMs production compared to the group on conventional detoxification and control group while the group on naltrexone showed statistically higher levels compared to the conventional detoxification (Tab. 2).

MDA levels presented a higher degree of lipid peroxidation in the group with heroin misuse compared to the other groups (Tab. 3):

The inter-group difference was statistically significant (df=3, F=5.295, p=0.003). The lipid peroxidation was higher in the heroin group compared to the group on conventional detoxification and control group (Tab. 3).

The correlation between d-ROMs and MDA was positive and statistically significant (r=0.647, p<0.001) (Fig. 1).

TAC was decreased in the heroin group compared to the other groups (Tab. 4).

Difference between the groups in TAC levels was statistically significant (df=3, F=8.296, p=0.001). The extracellular antioxidants were decreased in heroin abusers compared to the conventional detoxification and control group but they were also decreased in the group on naltrexon compared to the control group (Tab. 4).

Discussion

This study confirms previous findings that heroin (morphine) induces systemic OS and lowers the total antioxidant capacity. But, this is a first clinical study where the evaluation of systemic OS is estimated by measurement of d-ROMs. Their values are referred to hydroperoxide production as first line products of OH ± induced oxidation and are considered as an indicator of an early free radicals production and action. These results do not differ from others extensive studies, which are mostly experimental and also have demonstrated an increased production of ROS (14, 15). Previous clinical studies estimate the systemic OS indirectly, measuring the levels of lipid peroxidation product-MDA which represents a parameter of more advanced OS. MDA production is increased in the heroin group in our study and does not differ from previous findings. They match with the results of the studies where increased MDA was found in heroin addicts with a regular BMI (10) and also of the experimental and clinical studies where MDA increases with a prolonged administration time and increasing doses of heroin (6, 10). The studies, where MDA increases in addicts with active forms of viral hepatitis and nutritional deficits do not reflect the direct heroin toxic effects on OS (16), making an exclusion criterion in our study. Also, the values of d-ROMs and MDA in our patients are positively and statistically significantly correlated (r=0.647, p<0.001), manifesting their physiological complementarities, confirming devolved OS in chronic heroin addiction and usefulness of d-ROMs estimation in assessing OS in heroin addicts.

A possible mechanism of free radicals' increased generation and higher systemic OS in heroin use is a disturbance of central and peripheral adrenergic tone in chronic opioid addiction. An increased activity of tyrosine synthetase (17), adrenaline (18), were found in heroin addicts. Addicts on methadone maintenance program showed a higher urine metanephrines levels (19). Lowering of dopamine, noradrenaline and adrenaline levels in chronic heroin abuse with a significant rise in an acute morphine administration together with a lowered peripheral synaptic turn-over (DOPA/DA). Reserpine administration results in a significant decrease in catecholamines and amines levels by blocking the morphine effects on adrenergic system (20). Higher levels of normetanephrines were found in addicts with short duration of heroin misuse (21). These metabolites are important participants in heroin pharmacokinetic properties and action. Its toxicity is developing mainly due to the catechol/amines' auto-oxidation and generating of oxidizing toxic products: o-quinones and semiquinones in presence of trace metals, with consecutive free radical production, DNA and protein oxidation (22, 23).

The group on conventional detoxification achieved decreased levels of d-ROMs and lipid peroxidation compared to heroin abusers in our study. There is not a significant difference in MDA production between the group on detoxification and control group. Abating heroin and stabilizing the vegetative signs of withdrawal provides a decrease of an advanced systemic OS. Our results are in agreement with previous studies where withdrawal reactions induce and even increase the lipid peroxidation (24) what needed to be controlled. Abating aggravation of withdrawal syndromes in conventional detoxification with high doses of antioxidants (vitamin C and vitamin E) also indirectly confirms the arising OS in withdrawal syndrome (25). In an experimental study, restrain of OS and even alleviate of withdrawal syndrome in heroin-administered mice was achieved by administration of exogenous antioxidants (ascorbic acid, uric acid, glutathione, manitol, quercetin and resveratrol), confirming the development of OS in heroin induction and withdrawal (6). The control of adrenergic tone as a source of free radical production in our study was realized by chlorpromazine, which blocks D1 and D2 central receptors and peripheral α1 and α2 receptors (26). Additional antioxidant property of adrenergic block-

ing in opioid use is experimentally showed when propranolol inhibits morphine-induced superoxide production and apoptosis (27) and chlorpromazine antioxidant properties by lowering the OH^- levels in vitro (28). Complementary control of withdrawal syndromes' induced OS is provided by administration of diazepam which enhances the effects of GABA at receptors inducing inhibitory action and modulating dopaminergic, adrenergic, cholinergic and serotonergic activity, partially contributing to abating peripheral alpha adrenergic effects of the withdrawal syndrome (29, 30) and experimentally blocking peripheral benzodiazepine receptors, although not complexly studied, suppress the oxidative stress in the activated cells (neutrophils) (31). Use of these medications in our study probably influences a decreasing level of systemic OS, implicating a better antioxidant homeostasis in absence of heroin administration and catecholamine distress.

Our results showed a significantly decreased TAC in the heroin group. Lowering of the extracellular antioxidants is due to neutralization of an increased free radical production and lipid peroxidation in the heroin group. Our findings do not differ from the experimental study, where the estimation of an extracellular antioxidant capacity during heroin stimulation was performed by the measurement of TAC (6). Results from other studies showed a decreased antioxidant status in heroin addicts too, but confirmed decreased levels of each antioxidant (vitamin C, vitamin A, vitamin E) (9, 10) and antioxidases: catalasa, superoxide dismutaza, glutathione peroxidasa (10) using the HPLC methods. Lowering of antioxidant defense in heroin addiction in some studies (6, 9, 10) was related to the time of administration and/or dose of heroin, which was not analyzed in our study because of homogeneity of the groups. The restoration of TAC in the group on conventional detoxification in our study is explained by stopping the use of heroin and controlling the withdrawal syndromes, as sources of an increased OS.

This study is also the first that evaluate OS in naltrexone detoxification. Despite expecting better control of OS and withdrawal syndrome, naltrexone group showed statistically significant higher levels of d-ROMs compared to conventional detoxification that is not followed by an increased MDA production. We think that this effect is due to an early inclusion of the naltrexone patients in the study (first-third day) and very slow achievement of steady and sufficient naltrexone plasma concentration (5 ng/ml). These was confirmed in the study where a very unstable plasma levels of the naltrexone in first 3 days (32) were shown and a longer period for stabilization of peripheral adrenergic tone under naltrexone in other clinical study for naltrexone type of detoxification (33). This way, naltrexone group had good but not enough stabilized adrenergic tone followed by an increase of only d-ROMs' values as an early parameter of oxidative stress. The higher OS is followed by an increased utilization of antioxidants in neutralizing free radical production evaluated with statistically significant lower TAC in naltrexon group compared to controls. Also, a small number of patients included in this group, due to the price of the medication, may contribute to these results.

Smoking cigarettes was noted in all the patients (and controls) with the consumption about 20–30 cigarettes a day. In young populations under 30 years old, like the patients in our study, with satisfactory nutritional status and referent BMI, studies report not a significant influence of smoking on antioxidant status (34). Also, the lipid peroxidation levels, measured by TBARS reactions, as in our study, is not significantly influenced by smoking cigarettes, especially in young people with a good nutrition (35).

In conclusion, the results of our study suggest that long-term heroin abuse induces oxidative stress with a reduction of total antioxidant capacity. Abating heroin and introduction of detoxification methods decrease the free radical production and enable restoration of the extracellular antioxidant capacity in normally feeding patients. Dopaminergic activation and increased autooxidation of catecholamines is a possible source of higher free radical production and one of the mechanisms of heroin toxicity in chronic heroin misuse.

References

1. Patel J, Manappan N, Bhat R. Role of oxidative stress and heme-oxygenase activity in morphine-induced glomerular epithelial cell growth. Amer J Pysiol Renal Physiol 2003; 285: 861—869.

2. Zhang YT, Zheng QS, Pan J et al. Oxidative damage of biomolecules in mouse liver induced by morphine and protected by antioxidants. Basic Clin Pharmacol Toxicol 2004; 95 (2): 53—58.

3. Di Bello MG, Masini E, Ioannides C et al. Histamine release from mast cells induced by the metabolic activation of drugs of abuse into free radicals. Inflamm Res 1998; 47 (3): 122—130.

4. Kreigstein AR, Shungu DC, Millar WS et al. Leukoencephalopathy and raised brain lactate from heroin vapor inhalation „chasing the dragon"). Neurology 1999; 53: 1765—1773.

5. Slotkin TA, Seidler FJ, Yania J. Heroin neuroteratogenicity: targeting adenylil cyclase as an underlying biochemical mechanism. Brain Res Dev 2001; 132 (1): 69—79.

6. Bo Xu, Wang Z, Gang L et al. Heroin-administered mice involved in oxidative stress and exogenous antioxidant-alleviated withdrawal syndrome. Basic Clin Pharmacol Toxicol 2006; 99: 153—161.

7. Konstatinopolskii MA, Pirozhkov SV, Soloveva et al. The withdrawal syndrome and lipid peroxidation during the crhonica administration of narcotic analgesics to rats. Eksp Klin Farmakol 1992; 55 (1): 21—24.

8. Panchenko LF, Pirozhkov SV, Nadezhdin VIU, Usmanova NN. Lipid peroxidation, peroxyl-radical-scavering system of plasma and liver and heart pathology in adolescence heroin users. Vopr Med Khim 1999; 45 (6): 501—506.

9. Nazral Islam SK, Hossain KJ, Ahsan M. Serum vitamin E, C and A status of drug addicts undergoing detoxification: influence of drug habit, sexual practice and lifestyle factors. Europ J Clin Nutr 2001; 55: 1022—1027.

10. Zhou JF, Yan XF, Ruan ZR et al. Heroin abuse and nitric oxide, oxidation, peroxidation, lipoperoxidation. Biomed Environm Sci 2000; 13: 131—139.

11. Gutteridge JM. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem 1995; 41: 1819—1828.

12. http://www.nida.nih.gov/Diagnosis-Treatment.

13. Cesarone MR, Belcaro G, Carratelli M et al. A simple test to monitor oxidative stress. Int Angiol 1999; 18 (2): 127—130.

14. Sharp BM, Keane WF, Suh G et al. Opioid peptides rapidly stimulate superoxide production by human polymorphonuclear leukocytes and macrophages. Endocrinology 1985; 117: 793—795.

15. Oliveira MT, Rego AC, Morgadinho MT et al. Toxic effects and stiumulant drugs on undifferentiated PC 12 cells. Ann NY Acad Sci 2002; 965: 487—496.

16. Heathcote J, Taylor KB. Immunity and nutrition in heroin addicts. Drug Alcohol Depend 1981; 8: 245—255.

17. Guitart X, Hayward M, Nisenbaum LK. Identification of MARPP-58, a morphine- and cyclic AMP-regulated phosphoproteinof 58 kDa, as tyrosine hydroxylase: evidence for regulation of its expression bychronic morphine in the rat locus coeruleus. J Neurosci 1990; 10 (8): 2649—2659.

18. Kienbaum P, Heutert T, Michel CM et al. Chronic mu-opioid receptor stimulation in humans decreases muscule sympathic nerve activity. Circulation 2001; 103: 850—855.

19. De Leon-Jones FA, Davis JM, Inwang EE et al. Excretion of catecholamine metabolites during methadone maintenance and withdrawal. Arch Gen Psychiat 1983; 40 (8): 841—847.

20. Rabadan JV, Milanes MV, Larden ML. Effects of chornic morphine treatment on catecholamines content and mechanical response in the rat hearts. J Pharmcol Exp Therp 1997; 280: 22—37.

21. Schildkraut JJ, Meyer RE, Orsulak PJ et al. The effects of heroin on catecholamine metabolism in man. Natl Inst Drug Abuse Res Monogr Ser 1975; 3: 137—145.

22. Smythies J. Redox aspects of signaling by catecholamines and their metabolites. Antioxid Redox Signal 2000; 2 (3): 575—583.

23. Kovacic P. Unifying mechanism for addiction and toxicity of abused drugs with application to dopamine and glutamate mediators: electron transfer and reactive oxygen species. Med Hypothesis 2005; 65: 90—96.

24. Konstatinopolskii MA, Pirozhkov SV, Soloveva et al. The withdrawal syndrome and lipid peroxidation during the crhonica administration of narcotic analgesics to rats. Eksp Klin Farmakol 1992; 55 (1): 21—24.

25. Evangelou A, Kalfakakou V, Georgakis P et al. Ascorbic acid (vitamin C) effects on withdrawal syndrome of heroin abusers. In vivo 2000; 14: 363—366.

26. Laordeni ML, Fuartes G, Gonzgulez-Cuello A, Milangos MV. Changes in catecholaminergic pathways innervating paraventricular nucleus and pituitary-adrenal axis response during morphine dependence: implication of alfa(1)- and alfa(2)-adrenoreceptors. J Pharmacol Exp Ther 2000; 293 (2): 578—584.

27. Bhat RS, Bhaskaran M, Mongia A, Hitosugi N, Singhal PC. Morphine induced apoptosis: oxidative stress and strategies for modulation. J Leukoc Biol 2004; 75 (6): 1131—1138.

28. Jeding I, Evans PJ, Akanmu D et al. Characteriztion of the potential antioxidant and pro-oxidant action of some neuroleptic drugs. Biochem Pharmacol 1995; 49 (3): 359—365.

29. Gongulez-Cuello A, Sguncez L, Herngundez J et al. Phosphodiesteraze 4 inchibitors, rolipram and diazepam block the adaptive changes observed during morphine withdrawal. Europ J Pharmacol 2007; jun 9.

30. Suzuki T, Tsuda M, Narita M et al. Diazepam pretreatment suppresses morphine withdrawal signs in mouse. Life Sci 1996; 58 (4): 349—357.

31. Rajtar G, Zolkowska D, Kleinrok Z. Effect of diazepam and clonazepam on the function of isolated rat platelet and neutrophil. Med Sci Monit 2002; 8 (4): 37—44.

32. McDonald T, Berkowitz R, Hoffman WE. Plasma naltrexone during opioid detoxification. J Addict Dis 2000; 19 (4): 59—64.

33. Macedo TR, Relvas J, Fontes Ribeiro AC et al. Plasma catecholamines during ultrarapid heroin detoxification. Ann NY Acad Sci 2000; 914: 303—310.

34. Lykkesfeldt J, Christen S, Wallock LM et al. Ascorbate is depleted by smoking and repleted by moderate supplementation:a study in male smokers and nonsmokers with matched dietary antioxidant intake. Amer J Clin Nutr 2000; 71 (2): 530—536.

35. Ayaori M, Hisada T, Suzukawa M et al. Plasma levels and redox status of ascorbic acid and levels of lipid peroxidation products in active and passive smokers. Environ Health Perpect 2000; 105—108.

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