

THE IMPORTANCE OF VANILLYLMANDELIC ACID DETERMINATION IN OPIATE USERS

VAŽNOST ODREĐIVANJA KONCENTRACIJE VANILMANDELIČNE KISELINE KOD LJUDI KOJI KORISTE OPIJATE

Danijela Janičević-Ivanovska¹, Aneta Spasovska-Trajkovska², Branko Stefanovski²,
Slavica Subeska-Stratrova³, Jasmina Mecevska Jovcevska¹

¹University Clinic for Clinical Biochemistry

²University Clinic for Psychiatry

³University Clinic for Endocrinology, University Clinical Center, Skopje, R. Macedonia

Summary: Although knowledge about the etiology of heroin dependence is rather poor, it is known that the influence of opiates on the opioid, adrenergic and indirectly of GABA on the dopaminergic receptors leads to changes of catecholamine levels in brain structures, which are supposed to be essential in explaining the etiology of the opioid dependence. It is well-known that by analyzing catecholamine, we get vanillylmandelic acid (VMA), which is found in the urine as a final product. Thus, by an indirect determination of VMA it is also possible to define the catecholamine concentration in the brain, which is the aim of this study. This prospective study included 51 dependent heroin users divided into 3 groups, depending on the length of the medical treatment with a conventional detox method (without treatment, second day of the treatment, and after the 10th day of treatment) as well as a control group consisting of 20 healthy subjects. We used the Pissano method chromatographic-spectrophotometric determination – for estimating the level of vanillylmandelic acid, and a scale for defining the severity of symptoms of the withdrawal syndrome (WS). The results showed that the highest average values of the urinary level of VMA were found in the subjects in withdrawal crisis with a high WS scale score in comparison with the other examined groups (statistically significant) as a result of the high adrenergic level. The average score on the scale of depression was high in the subjects in withdrawal crisis where we also found low values of the VMA urinary level. Being aware of the neurobiology of heroin dependence is of great importance for finding new pharmacological treatments for heroin addiction.

Keywords: vanillylmandelic acid, opioid addiction, withdrawal syndrome

Kratak sadržaj: Siromašna su saznanja o etiologiji opioidne zavisnosti, ali se ipak smatra da opijati deluju na opioidne adrenergične i po indirektnom putu preko GABA na dopaminske receptore, dovodeći do promene kateholaminskih nivoa u moždanim strukturama, za koje se pretpostavlja da su važne za objašnjenje etiologije opioidne zavisnosti. Poznato je da se razgradnjom kateholamina dobija vanilmandelična kiselina (VMA), kao poslednji produkt u urinu, pa postoji mogućnost da se određivanjem njene koncentracije indirektnim putem proceni i koncentracija kateholamina u mozgu, što je i cilj ovog rada. Prospektivna studija obuhvata ukupno 51 heroinskih zavisnika na detoksikacionom programu, podeljenih u tri grupe u zavisnosti od medicinskog tretmana – prva grupa su heroinski zavisnici (bez tretmana), druga grupa zavisnici koji su započeli tretman dva dana ranije, treća grupa heroinski zavisnici koji su pre 10 dana počeli tretman (stabilizanti), i kontrolna grupa od 20 zdravih ispitanika. Tokom istraživanja korišćen je metod Pissano – hromatografsko spektrofotometrijsko određivanje nivoa VMA, i skala za procenu jačine simptoma apstinencijalne krize (WS). Dobijeni rezultati ukazuju da najveće prosečne vrednosti urinarnih nivoa VMA imaju heroinski zavisnici koji su u apstinencijalnoj krizi gde se vidi i visoki skor WS skale u odnosu na druge grupe, kao rezultat visokog adrenergičnog nivoa. Prosečni skor skale depresivnosti je visok kod zavisnika (10 dana od početka tretmana gde postoji nizak nivo VMA). Poznavanje neurobiologije opioidnih zavisnika, od velikog je značaja za pronalaženje novih farmakoloških tretmana za njihovo lečenje.

Ključne reči: vanilmandelična kiselina, opioidna zavisnost, apstinencijalni sindrom

Address for correspondence:

Danijela Janičević-Ivanovska
University Clinic for Clinical Biochemistry
University Clinical Center, Skopje, R. Macedonia
Mobil: +389 70 678 727
e-mail: djanicevic@yahoo.com

Introduction

The term opiates is used for psychoactive substances as well as for the half-synthetic drug heroin, which is produced from poppy seeds. Intake of heroin into the human organism via nasal snorting, by inhaling its vapors, that is, heating heroin on a piece of foil, and intravenous injection have influence on the intensity and character of the negative effects (1). High liposolubility of heroin enables rapid heroin crossing of the blood-brain barrier and the consequent metabolic activation of CNS. Heroin is hydrolyzed to 9-monoacetylmorphine that enters the CNS and has the same pharmacological characteristics as heroin. It is further deacylated to morphine, which remains present and potent in the CNS long after heroin elimination from the organism (2).

Opioid dependence is a result of the effect of opioid adrenergic receptors and indirectly of GABA effect on dopamine receptors that lead to changes of catecholamine levels in the brain structures, which indirectly assess the heroin effects on the CNS (3, 4).

It is also assumed that the dosage is not the unique factor for the changes in the organism that appear after heroin use, but the mode of application is essential, too (high doses by intravenous injection lead to fatal outcome) (5). All these are important for further therapeutic procedures (6).

The dose and time duration of opiate application are in positive correlation with the clinical picture of opiate dependence along with the catecholamine levels in the brain that are indirectly precisely assessed through their urine catecholamine products – vanillylmandelic acid (VMA). By catecholamine degradation under the effect of the enzyme catecholamine-*o*-methyl-transferase (COMT) VMA is obtained, which is found in the urine as a final product (7).

VMA values reflect the real catecholamine production in the brain structures. Thus, determination of VMA in urine is very important in order to assess the indirect heroin effect on catecholamine production in the brain (8–10). Some studies have reported that by the effect of heroin on brain structures, changes in the level of catecholamine, that is, catecholamine concentration in the organism are also provoked. Heroin arouses pleasant feelings, but on the other hand, withdrawal crisis may appear as well as the addiction syndrome (11).

In this paper, the presence of VMA that is found as a final product in the urine of opioid-dependent drug users was determined, as well as the indirect VMA influence on CNS during a ten-day period in patients undergoing a detoxification program.

Material and Methods

The investigation included 51 subjects of both sexes, aged between 18 and 35 years (mean age 22

years), who signed a written consent for their participation in this investigation. The patients were divided into three groups depending on the duration of detoxification treatment. Detoxification was done with benzodiazepines and parenterally with vitamins from the B-complex group.

Sixteen (22%) patients who are regular users of opiate agents (heroin) without treatment (first day of heroin interruption) were named group A; 18 (35.4%) patients who were undergoing detoxification treatment on day 2 (second day after heroin interruption, when withdrawal crisis appears) were named group B; 17 (23.9%) patients who were undergoing detoxification treatment on day 10 (tenth day after heroin interruption and they were in a stable condition) were named group C.

The control group consisted of 20 healthy subjects – blood donors, with normal laboratory findings, with similar distribution by sex and age as the examined patients. This group was named H.

VMA was determined in all subjects. 24-hour urine was collected and acidified with 10 mL HCl. VMA was determined using the Pissano method – chromatographic-spectrophotometric determination (12). VMA reference values are 9.0–34.6 $\mu\text{mol}/24\text{h}$.

The results obtained were statistically analyzed with the SPSS 10 for Windows statistical program. A method for descriptive statistical analyses, Student's *t*-test was used for the analysis. Values of $p < 0.005$ were considered to be statistically significant.

Results

By applying the *t*-test for independent samples we tested the differences in the average length of heroin dependence between the three examined groups. Subjects from group B had significantly longer opioid addiction in comparison with group A ($p < 0.05$); significantly longer in comparison with those in the stabilization phase, group C ($p < 0.001$), while the length of opioid addiction in group A was insignificantly longer in subjects on street heroin (group A) than in subjects who were in the detoxification program for 10 days, group C ($p > 0.05$). The highest average VMA values were found in group B (50.9 ± 8.7), lower in group A (38.8 ± 9.2), the lowest in group C (33.2 ± 6.1), and in the control group H (32.3 ± 1.2).

Table I Mean values of VMA urinary level by groups.

VMA, $\mu\text{mol}/\text{L}$	N	Mean	Min	Max	SD
Group B	18	50.95 \uparrow	37.60	62.70	8.66
Group A	16	38.85 \uparrow	27.30	62.50	9.19
Group C	17	33.22	22.10	43.20	6.08
Control group H	20	32.34	30.00	34.00	1.18

Table II Distribution of values of the severity of withdrawal symptoms (WS) by groups.

WS	Group B		Group A		Group C	
	Number of patients	Number of patients (%)	Number of patients	Number of patients (%)	Number of patients	Number of patients (%)
1 – moderate	0	0	12	75.00	17	100.00
2 – mild	8	44.44	4	25.00	0	0
3 – severe	10	55.56	0	0	0	0
Total number of patients	18	100.00	16	100.00	17	100.00

Group B had significantly higher average values of VMA (50.09 ± 8.7) in comparison with the control group (32.3 ± 1.2) ($p < 0.001$). Group A had also significantly higher values (38.8 ± 9.2) ($p < 0.001$), whereas the VMA value in group C (33.2 ± 6.1) did not significantly differ in comparison to the control group H.

Deviations from the normal values ($\leq 34 \mu\text{mol/L}$) with regard to increased average values were registered in the addicts from group B and group A. The results of the tested differences of average VMA serum concentrations between the examined groups are presented in *Table I*.

The subjects from group B had significantly higher average values of the analyzed acid in comparison with the other examined groups ($p < 0.01$). Heroin addicts from group A had significantly higher average values of VMA in comparison with both addicts from group C ($p < 0.05$) and control subjects ($p < 0.01$).

Withdrawal crisis according to the withdrawal scale scores can be mild, moderate and severe. Among addicts in the acute phase (group B), there were no subjects with moderate form of withdrawal crisis, while more than 50% of these subjects had severe degrees of withdrawal crisis. Among street heroin-dependent users (group A), there were none with a severe degree of withdrawal crisis, 75% had a moderate degree and 25% a mild degree that was the result of abstinence. All 17 patients in the stabilization phase (group C) had a moderate degree of withdrawal crisis. Obtained results are shown in *Table II*.

Discussion

Some studies have reported increased urinary elimination of the catecholamine metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) in the majority of heroin addicts in comparison with a control group (13). In the study conducted by Roffman et al. (14), the level of catecholamines, that is, their urinary metabolite VMA was monitored and it was proved that VMA increase was analogous to heroin administration in the group of examined drug

users (chronic heroin addicts) and it was bigger in comparison with the control group. Also, the investigation conducted by Macedo et al. (15) showed that the plasma level of conjugated catecholamine was increased in heroin addicts.

In our study we examined the urinary level of VMA and the results obtained showed that street heroin-drug users from group A had significantly higher average VMA values than the control group of subjects.

Literature data have confirmed a positive relation between the length of opioid addiction and changes in the catecholamine level during opiate abuse (16).

There is a positive correlation between VMA concentrations in urine and the length of opioid addiction. The value of coefficient P has shown that the relation ranges between moderate and high in addicts in severe withdrawal crisis. Concentration of vanillylmandelic acid in urine increases proportionally with the length of opioid addiction; long-term addiction is associated with higher concentrations of this acid. The patients from group B who were in the second day of the detoxification program were found to be in a more severe withdrawal crisis and they were longer-term addicts. This relation, that is correlation, is strong and is statistically significant.

During early withdrawal crisis an increase in catecholamine secretion was noticed monitored by increase of the excretion of urinary MHPG and VMA (17). We have also examined the urinary level of VMA and the results obtained pointed to significantly highest average values of VMA in comparison with the remaining groups and the control group. The subjects in acute withdrawal crisis had significantly higher average values of the analyzed acid when compared to the other groups.

According to some researchers the urinary level of VMA in the first days of withdrawal crisis in opioid addicts is increased and then stabilized to the normal level (18). Results presented in the study of Alec Roy demonstrated that there were no significant differences of correlation between the plasma level of VMA in

abstinent heroin dependent addicts when compared with the control group and in relation to the number of days of abstinence (until the 28th day of abstinence and after the 28th day of abstinence) (19).

In our subjects from the group of stable examinees (abstinent patients after the 10th day of detoxification), a significant decrease of the urinary level of VMA was proved in comparison with the other groups and the control group (healthy population). The obtained average values of VMA in addicts in the stabilization phase did not differ significantly from those in the healthy subjects, that is, the tested differences between them were statistically insignificant.

In the programs for detoxification with phenothiazines and benzodiazepines the most commonly used protocol for detoxification treatment is based on the grounds that in opioid addiction there is presynaptic dopaminergic stimulation, and thus the most frequently applied medications are those that block the dopamine release – dopamine antagonists (20, 21).

According to some studies the severity of withdrawal crisis is in direct correlation with the applied dose, intravenous injection and length of opioid addiction (22). The patients in their second day of the detoxification program experience a more severe withdrawal crisis if they are long-term addicts. Usually, long-term heroin use is related to increase of the dose, which leads to emphasizing the withdrawal signs and symptoms (as was the case with the group B in our study). Opioid-dependent addicts from the group A had increased values when compared with the controls. The VMA values in the subjects from the group C did not significantly differ from group H,

which points to the fact that these values are a result of successful detoxification and can be used as a predictor for further successful treatment.

Testing differences of the withdrawal scale scores in all examined relations were highly statistically significant ($p < 0.01$). Therefore, a conclusion could be drawn that subjects in the acute withdrawal crisis have significantly higher scores on the withdrawal scale than the patients on the first day and those in the stabilization phase – the 10th day of treatment (23, 24). Also, patients on their first day of treatment had significantly higher scale scores than those in the stabilization phase (the 10th day of treatment); however, this statistical significance was smaller than the significance registered between the patients in acute state and the remaining two groups.

The results of this clinical study have confirmed the expected conclusions, having in mind the numerous cited references.

In conclusion, opioid addiction is characterized by an increased VMA value in the urine, especially in the withdrawal crisis that is characterized by significantly high VMA values and is of key importance for the assessment of the severity of withdrawal crisis. The detoxification program conducted on heroin-dependent addicts enables normalization of the VMA values on the 10th day of treatment. Our notions imply that VMA determination is indispensable for the indirect assessment of opioid addiction and for the course and outcome of the treatment. This means that opioid addiction as a diagnosis should be taken into account seriously and adequately treated for the well-being of opioid-dependent drug users.

References

- Zquierdo Patron M, Martinez-Moratalla J, Gonzales Valladers G. Broncho-spasm from heroin inhalation. *An Med Interna* 2001; 18: 165.
- McFarland K, Ettenberg A. Haloperidol does not attenuate conditioned place preferences or locomotor activation produced by food or heroin-predictive discriminative cues. *Pharmacology, biochemistry, and behavior* 1999; 62: 631–41.
- Lawford B, Young R, Noble E, Sargent J, Rowel J. The D2 Dopamine Receptor A1 Allele and Opioid Dependence: Association With Heroin Use and Response to Methadone Treatment. *American Journal of Medical Genetics Neuropsychiatric Genetics* 2000; 96: 592–8.
- Cousins MS, Roberts DC, De Wit H. GABA (B) receptor agonists for the treatment of drug addiction: a review of recent findings. *Drug and alcohol dependence* 2002; 65: 209–20.
- Platt DM, Rowlett JP, Spealman RD. Discriminative, stimulus effects of intravenous heroin and its metabolites in rhesus monkeys: opioid and dopaminergic mechanisms. *Journal of pharmacology and experimental therapeutics* 2001; 299: 760–7.
- Giannini J. An Approach to Drug Abuse. *Intoxication and Withdrawal*. *Am Fam Psych* 2000; 661: 2763–74.
- Cadlovski G. *Nevroleptici*. *Koniks* 1999; 12–50, 86–92.
- De Maio D, Caponeri MA, Cicchetti V, Mellado C, Scieghgi G. Sulpiride and extrapyramidal syndromes in chronic heroin addiction. *Neuropsychobiology* 1978; 4: 36–9.
- Gerra G, Zaimovic A, Zambelli U, Delsignore R, Baroni MC, Laviola G. Neuroendocrine correlates of depression in abstinent heroin-dependent subjects. *Psychiatry research* 2000; 96: 221–34.
- The effects of heroin on catecholamine metabolism in man. *Natl Inst Drug Abuse Res Monogr Ser*. 1985; 137–45.

11. EMCDDA. Evaluating the treatment of Drug Abuse in the European Union. Luxemburg: Office for Official Publications of the European Communities, 1999: 13–28.
12. Pissano J, Crout R, Abraham D. Determination of 3-methoxy-4-hydroxymandelic acid in urine. *Clin Chim Acta* 1962; 7: 277–84.
13. Schildkraut J, Meyer RE, Orsulak PJ, Mirin SM, Roffman M, Platz PA, Grab E, Randall ME, McDougale M. Catecholamine metabolism during heroin use. *Am J Psychiatry* 1997; 134: 534–7.
14. Roffman M, Platz PA, Grab E, et al. The effects of heroin on catecholamine metabolism in man. *Nat Inst Drug Abuse* 1995 (3): 137–45.
15. Macedo TR, Riberio A, Morgandino T, et al. Influence of concurrent heroin and cocaine abuse on the adrenergic and serotonergic system in man. *Annals of the New York Academy of Sciences* 1998; 844: 208–13.
16. Kish SJ, Kalasinky KS, Derkach P, et al. Striatal dopaminergic and serotonergic markers in human heroin users. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology* 2001; 24 (5): 561–7.
17. Hunyor S, Hansson L, Harrison T, Hoobler S. Effects of Clonidine Withdrawal: Possible Mechanisms and Suggestions for Management. *Br Med J* 1973; 28: 2 (5860): 209–11.
18. Warner-Smith M, Darke S, Lynskey M, Hall W. Heroin overdose: causes and consequences. *Addiction* 2001; 96; 11: 3–25.
19. Roy A, Berman J, Williams R, Kuhn C, Gonzalez B. Higher Levels of CSF Homovanillic Acid in Recently Abstinent Cocaine-Dependent Patients. *Am J Psychiatry* 2002, 159: 1053–5.
20. Collier H, Francis D, Roy A. Opiates, cyclic nucleotides, and xanthenes. *Advances in biochemical psychopharmacology* 1976; 15: 337–45.
21. De Vries TJ, Schoffelmeer AN, Binnekade R, Raaso H, Vanderschuren LJ. Relapse to cocaine- and heroin-seeking behavior mediated by dopamine D2 receptors is time-dependent and associated with behavioral sensitization. *Neuropsychopharmacology* 2002; 26: 18–26.
22. EMCDDA. Extended annual report on the state of the drugs problem in the European Union 1999. Luxemburg: Office for Official Publications of the European Communities, 1999: 9.
23. Oliveira MT, Rego AC, Morgadinho MT, Macedo TR, Oliveira CR. Toxic effects of opioid and stimulant drugs on undifferentiated PC 12 Cells. *Annals of the New York Academy of Sciences* 2002; 965: 487–96.
24. Ghaffari-Nejad A, Sabih K. The relationship between heroin withdrawal signs and symptoms and existence of depression. *Arch Iranian Med* 2005; 8: 115–8.

Received: January 10, 2009

Accepted: April 11, 2009