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CAN AN INCREASE OF NITRIC OXIDE METABOLITES CONCENTRATION AFTER FIRST FOUR WEEKS OF ABSTINENCE PREDICT ALCOHOL RELAPSE DURING THE NEXT FIVE MONTHS?

Jacek Budzyński[1], Maria Kłopocka[1], Maciej Świątkowski[1], Marcin Ziółkowski[2], Grzegorz Pulkowski[1], Ewa Kopczyńska[3]

[1]Department of Gastroenterology, Vascular Diseases and Internal Medicine, Ludwik Rydygier Medical University in Bydgoszcz, Poland

[2]Department of Psychiatric Nursing, Addiction Treatment Unit, Department of Psychiatry, Ludwik Rydygier Medical University in Bydgoszcz, Poland

[3]Department of Pathobiochemistry and Clinical Chemistry, Ludwik Rydygier Medical University in Bydgoszcz, Poland

Abstract – BACKGROUND. Nitric oxide (NO) is involved in a pathogenesis of alcohol dependence and affects the course of withdrawal syndrome. The aim of a study was to compare NO metabolites plasma levels in alcohol dependent patients (AD), who continued abstinence for 6 months and in those who relapsed during this period. INVESTIGATED GROUP AND METHODS. In 26 males with alcohol dependence (AD) who had finished their alcohol misuse no longer than 14 days before the study start NO metabolites plasma levels were determined at the baseline and twice during the observation period: at 4 weeks and at a 6-month follow-up, using the colorimetric method. RESULTS: At the 6-month follow-up AD subjects who relapsed to alcohol drinking (n=9, i.e. 35%) had significantly higher levels of NO metabolites than had those who remained abstinent (n=17, or 65%). However, the difference was found to be due to an increase in the in relapsed patients' mean NO metabolites level noted as soon as after four weeks of abstinence. An increase in the NO metabolites level or a decrease by no more than 4,4 mcmol/l was observed in 100% of the relapsed alcoholics and only in 53% of AD patients who remained abstinent after six moths (p=0.023). Alcohol drinking relapse was predicted in logistic regression by the cut-off value of delta nitrites concentration amounting to – 4,4 mcomol/l after 4-week abstinence. CONCLUSION: This observation suggests that an increase or a slight decrease in NO metabolites plasma concentra-

tion following 4-week abstinence may be a predictor of alcohol drinking relapse during the next five months, but this requires further study.
Key words: nitric oxide, alcohol dependence, relapse.

Czy zwiększenie stężenia metabolitów tlenku azotu po czterech tygodniach abstynencji może prognozować przerwanie abstynencji w ciągu kolejnych pięciu miesięcy?

Streszczenie – Tlenek azotu (NO) uczestniczy w mechanizmach uzależnienia od alkoholu oraz moduluje przebieg zespołu abstynencyjnego. W pracy dokonano retrospektywnego porównania stężeń metabolitów NO u pacjentów z zespołem zależności alkoholowej (zza), którzy utrzymali półroczną abstynencję, z wartościami u chorych, którzy w tym okresie ją przerwali. MATERIAŁ I METODY: U 26 pacjentów z zza, którzy zakończyli picie alkoholu nie wcześniej niż przed 14 dniami, na początku badania, po 4 tygodniach i 6 miesiącach obserwacji oznaczono stężenie metabolitów NO. WYNIKI: Pacjenci, którzy nie utrzymali półrocznej abstynencji (n=9, 35%), po 6 miesiącach obserwacji mieli istotnie większe stężenie metabolitów tlenku azotu, niż chorzy, którzy nie przerwali abstynencji alkoholowej w tym okresie (n=17; 65%). Analiza retrospektywna wykazała jednak, że różnica między podgrupami była wynikiem zwiększenia stężenia metabolitów NO już po 4 tygodniowym okresie abstynencji, kiedy to wszyscy badani byli hospitalizowani. Zwiększenie stężenia metabolitów NO lub jego zmniejszenie nie więcej niż o 4,4mcmol/l obserwowano po 4 tygodniach u 100% pacjentów, którzy przerwali abstynencję i u 53% chorych, którzy nie pili alkoholu w ciągu półrocznej obserwacji (p=0,023). Zwiększenie stężenia tlenku azotu lub jego zmniejszenie z punktem odcięcia na poziomie 4,4mcmol/l były niezależnymi czynnikami predykcyjnymi przerwania abstynencji w ciągu 6 miesięcy obserwacji w regresji logistycznej. WNIOSEK: Zwiększenie lub niewielkie zmniejszenie syntezy tlenku azotu w ciągu pierwszych 4 tygodni abstynencji może być czynnikiem prognostycznym przerwania abstynencji w ciągu kolejnych 5 miesięcy obserwacji, jednak zjawisko to wymaga dalszych badań.

INTRODUCTION

Nitric oxide (NO) is a mediator regulating a number of human body functions. Its synthesis may be stimulated by many factors, including a relapse to alcohol drinking after 4-week abstinence. However, research results are ambiguous and show either no relationship or suppressive and even stimulating effect of alcoholic beverages on NO synthesis both after acute and chronic administration (1, 9, 11, 12, 23, 26, 29, 34). Moreover, some reports suggest that NO may be involved in molecular mechanisms for ethanol-induced liver disease (5, 18, 24) as well as substance abuse and dependence upon opioids, ethanol, and such psychostimulants as cocaine, marihuana, nicotine (6, 17, 20, 21, 29, 30), and antenataly (8). Neural isoenzyme of nitric oxide synthase (nNOS) participated in the development of rapid tolerance to ethanol

(22) and morphine (13), while inhibitors of NOS modulated withdrawal from opioids, nicotine and ethanol, diminishing many signs of the withdrawal syndrome (31-33, 36).

In our paper a clinical observation is presented of changes in the plasma level of NO metabolites differing alcoholics who maintained abstinence over six months from those who relapsed to alcohol drinking within this period. In papers available to the authors no similar data have been reported.

SAMPLE AND METHODS

Data analysed in the paper were obtained from 26 alcohol dependent male patients who finished their alcohol misuse not longer than 14 days before the study start. Alcohol dependence was diagnosed according to the ICD-10 criteria. During the first four weeks of observation the patients were hospitalised in the Addiction Treatment Unit, Department of Psychiatry, the Ludwik Rydygier Medical University in Bydgoszcz (Poland). Their treatment consisted in training how to cope with alcohol craving. During hospitalisation their abstinence status was controlled: besides physical examination, there were tests for alcohol presence in the exhaled air, and for the blood level of biochemical markers of alcohol abuse (including the mean corpuscular volume – MCV, HDL cholesterol concentration, gamma-glutamyltransferase – GGT, aspartate aminotransferase – AST, alanine aminotransferase – ALT). The patients' relapse to alcohol drinking within the next 5 months following their discharge from the Addiction Treatment Unit was diagnosed on the basis of their medical history, the above-listed biochemical markers of alcohol abuse determined during their control visits, an objective familial interview, and medical documentation analysis. All the 26 subjects maintained abstinence over the first four observation weeks, but at the 6-month follow-up only 17 patients (65%) turned out to remain abstinent, while 9 (35%) relapsed during this period. The patients' mean age was 41.8±8.2 years, and their mean duration of alcohol dependence 19.4±7.6 years. They scored on the average 43.8±23.6 points on the Michigan Alcoholism Screening Test (MAST) and 25.6±7.2 points on the Short Alcohol Dependence Data (SADD). During the 90 days preceding their hospital admission they had drunk on the average 928.7±525.5 standard drinks (1 drink =1 oz of pure ethanol). In that period they had been consuming alcohol on the average for 52.9±22.9 days. All the patients were smokers both before and during the study.

Blood sampling for all the determinations (in morning, on an empty stomach), was carried out thrice: at the study beginning, at four weeks, and the 6-month follow-up. Biochemical markers of alcohol abuse were determined using the standard laboratory methods. The blood samples for NO measurements were refrigerated in -80 Celsius grad until determination was made. The NO plasma level at each visit was estimated as the mean of two plasma nitrite concentration determinations using the colorimetric method and the Nitric Oxide Colorimetric Assay manufactured by the Boehringer Mannheim, according to the instruction of the set producer. This assay consists in enzymatic reduction of nitrates to nitrites (nitrate reductase), followed by a spectrophotometric analysis

of total nitrites using the Greiss reagent (measured at 540 nm). This method of nitric oxide determination has a biochemical background and was used in the plasma NO metabolites level estimation in humans in other studies (7, 25).

All the subjects gave their informed consent to participate in this study, which was approved by the Local Ethics Committee of Ludwik Rydygier Medical University in Bydgoszcz. The investigation was in compliance with the Declaration of Helsinki for medical research.

In the statistical analysis including the unpaired Student's t-test, one- and two-way ANOVA with repeated measures, the Tukey's post hoc test, Fisher's exact test, as well as logistic regression equations, the statistical software STATISTICA PL 5.0 for Windows was used.

RESULTS

In whole group studied the mean plasma NO metabolites concentration did not change significantly within the 6-month observation period (Fig. 1). However, changes in the nitrite concentration in successive determinations were significantly related to the abstinence status at the 6-month follow-up (Fig. 2). In alcoholics who relapsed before the 6-month follow-up (n=9), the mean NO metabolites level had increased as early as after four weeks of controlled abstinence. Consequently, at the 6-month follow-up the abstinent alcoholics (n=17) had significantly lower concentration of NO metabolites than had those who failed to maintain abstinence (26,2±11,8 vs. 41,8±15,4 mcmol/l, respectively; p=0,015). In the whole group (n=26) the median difference between the plasma NO metabolites concentration measured at the study beginning and after the four weeks' abstinence was 2, with the 95% confidence interval (95% CI) ranging from -4.4 to 9.2 mcmol/l. Assuming the lower value of the 95% CI as a cut-off point for the change in the

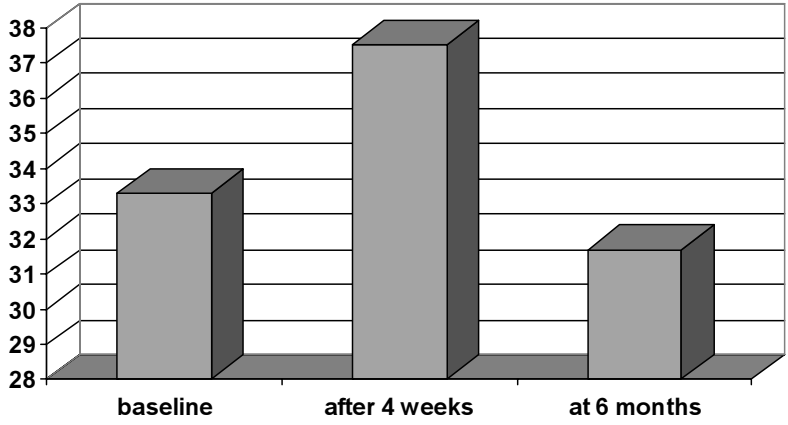

Fig. 1. Plasma nitric oxide metabolites' concentration at the study beginning, after four weeks and at a 6-month follow-up in the whole sample of alcohol dependent male patients (n=26).
Two-way ANOVA with repeated measures, F (2,42) = 3.45; p = 0.041

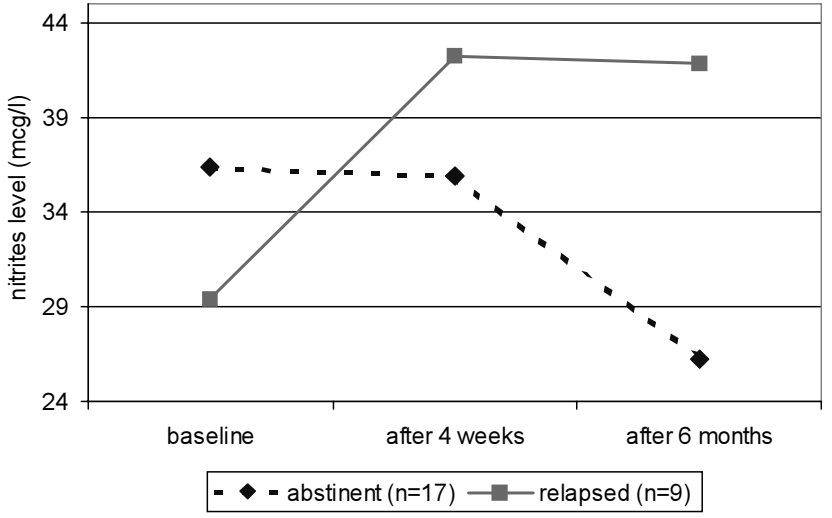

Fig. 2. Significant effect of the interaction between abstinence maintenance and the observation period duration on changes in the plasma nitric oxide metabolites (nitrites) concentration in male alcoholics during 6-month observation.

TABLE 1
Baseline demographic, clinical and biochemical characteristics of alcohol dependent patients divided by their abstinence status at the 6-month follow-up.

Characteristic	Abstinent pts (n = 17)	Relapsed pts (n = 9)	p =
Age (years)	40,6±8,5	44,1±7,1	0,30
SADD (score)	29,2±6,0	19,2±4,8	0,001
MAST (score)	48,5±7,0	37,0±18,5	0,27
Age of alcohol dependence onset (years)	22,0±6,6	24,7±6,1	0,34
Length of alcohol dependence (years)	18,8±7,8	19,4±7,4	0,84
Number of drinking days during 90 days before the study start	49,0±22,4	56,8±26,7	0,45
Number of standard drinks drunk during 90 days before the study start	989,6±578,9	824,3±506,4	0,49
Number of standard drinks drunk during 30 days before the study start	250,7±171,6	203,8±117,4	0,48
Mean daily nicotine dose (mg/day)	31,8±15,9	31,4±13,3	0,96
Mean daily tar dose (mg/day)	370,8±171,2	377,5±147,1	0,93
Systolic blood pressure (mmHg)	115,5±8,1	111,9±12,8	0,42
Diastolic blood pressure (mmHg)	76,3±7,9	76,9±8,0	0,88
BMI (kg/m2)	24,5±2,8	25,7±3,8	0,41
WHR	0,96±0,5	0,98±0,5	0,38
MCV (fl)	97,3±5,9	99,3±4,8	0,40
GGT (U/l)	89,9±58,1	75,8±33,5	0,80
AST (U/l)	24,1±18,8	26,4±7,6	0,73
ALT (U/l)	28,7±29,5	34,2±20,3	0,62
HDL cholesterol (mg/dl)	50,4±11,3	52,9±17,4	0,67

Abbreviations: SADD - Short Alcohol Dependence Data, MAST- Michigan Alcoholism Screening Test, BMI - body mass index, WHR- waist to hip ratio; MCV- mean corpuscular volume, GGT - gamma-glutamyltransferase, AST- aspartate aminotransferase, ALT- alanine aminotransferase, Data are presented as mean ± standard deviation; unpaired Student's t-test was used to determine statistical significance of differences between groups,

above-mentioned NO metabolites level (NO-delta), either an increase or a slight decrease (no greater than -4,4 mcmol/l – in the NO metabolites concentration was found in 100% of the relapsed alcoholics (n=9/9) and in 53% (n=9/17) of those who remained abstinent at the 6-month follow-up. The relationship between relapse before the 6-month follow-up and NO-delta value greater than -4,4 mcmol/l was significant according to Fisher's exact test (p=0,023) and had a relatively high positive power as evidenced by Kendall's tau coefficients (b=0.49; c=0.43). Moreover, logistic regression indicated that any change exceeding -4,4 mcmol/l in the NO metabolites concentration over four weeks of early abstinence was a significant predictor of relapse to alcohol drinking ($\chi 2$=8,6; p=0,0034). Apart from a lower SADD score in relapsed patients no other significant differences were found at baseline between abstinent and relapsed alcoholics as regards factors that might affect NO synthesis, including the amount of alcohol consumption, smoking status and liver condition (Tab. 1).

DISCUSSION

In this study changes in the NO metabolites concentration in 26 alcohol dependent males during six months' abstinence are reported. In our opinion the research findings raised two issues probably important for clinical practice. Firstly, alcoholics who relapsed within the 6-month follow-up were found to have a significantly higher level of NO metabolites at the 6-month visit than had those who maintained abstinence (Fig. 2). Besides, ANOVA indicated a significant effect of interaction of two factors: alcohol abstinence maintenance and the length of observation period on changes in the NO metabolites concentration (Fig. 2). An increase in the NO synthesis or release after alcohol drinking was previously reported (1, 9, 11, 29). However, as can be seen in Fig. 2, in our study the increase in the NO metabolites level of the relapsed alcoholics was noted as soon as after four weeks of abstinence, when all the subjects were hospitalised and their abstinence was controlled. This evidences that the NO metabolites increment was not due to alcohol drinking.

The relationship between NO metabolism and relapse to alcohol drinking found in our study may be explained in terms of central (cerebral) and peripheral (vascular) mechanisms. According to the literature, not only alcohol drinking, but also cessation of drinking may stimulate nitric oxide production in the brain sites involved in the expression of the withdrawal syndrome signs, although not all the symptoms in question result from the nitric oxide action (1). Moreover, Gerlach et al. reported findings of their post-mortem examination of the brains of alcoholics as compared to these of non-alcoholic individuals. In the former the nNOS protein expression was increased in the following regions: frontal cortex, the cingulate gyrus, the nucleus accumbens, the entorhinal cortex and the thalamus (11). On the other hand, treatment with a nitric oxide donor inhibited the anaesthetic effect of alcohol, blocked the effect of the NOS inhibitor on alcohol-induced anaesthesia and enhanced the severity of some signs of withdrawal from alcohol (1, 2, 35, 36). Moreover, administration of NOS inhibitors alone or in the combination with clonidine decreased the intensity of the opioid

withdrawal syndrome whose pathomechanism is similar to that of the alcohol withdrawal syndrome (1, 14, 17). However, the study by Spanagel et al. showed that the effect of nonselective NOS inhibitors on alcohol drinking was not mediated by neuronal isoenzyme of NOS (27). These observations imply that a cerebral increase in the NO synthesis or release may increase the alcohol withdrawal syndrome severity. This may facilitate relapse to alcohol drinking, which was indirectly observed in our study on the basis of the plasma NO metabolites level determination.

The peripheral mechanism of relationships between an excessive NO production during the early abstinence period and the failure to remain abstinent at the 6-month follow-up may result from the vasomotor effect of NO. It is involved in the regulation of vascular tonus, both directly and indirectly, via the central and peripheral neural systems (10). The excess of NO may lead to vascular dilatation, thus causing rush, tachycardia and sweating, i.e. typical withdrawal syndrome symptoms. Aggravation of these symptoms may make abstinence difficult to maintain, which is concordant with our results.

The second noteworthy finding of our study was that after the four weeks' abstinence a NO-delta value exceeding -4,4mcmol/l allowed to identify all the alcoholics who failed to remain abstinent at the 6-month follow-up. Predictive significance of this variable for relapse to alcohol drinking was confirmed in logistic regression. In the literature available to the authors no report concerning the importance of plasma NO metabolites level determination in the prognosis of drinking relapse could be found. So far the following factors have been reported as predictors of relapse to alcohol drinking: self-efficacy expectancy and short previous time in abstinence (35), depression after alcohol treatment (6), the Obsessive-Compulsive Drinking Scale score (OCDS) (4), the drinking-related locus of control scale (DRIE) and reasons for drinking (15).

The SADD questionnaire indicates alcohol dependence severity, and alcoholics with higher scores on the SADD had increased values of GTP and ALT activity (19). However, in our study patients who at the 6-month follow-up remained abstinent, at the study start had not only similar values of demographic and clinical features (including biochemical alcohol abuse markers), but also had even higher scores on the SADD (Tab. 1). Therefore, in their case the probability of a good outcome of antirelapse treatment was lower (28). Despite some limitations discussed below, this increases the probability that the differences between abstinent and relapsed alcoholics in the changes of the plasma NO metabolites concentration observed in our study after four weeks' abstinence may really affect mechanisms underlying the maintenance of abstinence over six months.

One of limitations of our study was a relatively small size of the compared subgroups (n=17 vs. 9). However, since the obtained intergroup differences in the NO metabolites level turned out to be statistically significant, this limitation paradoxically increased the probability of our results repeatability in a larger sample. Secondly, in our study during the outpatient follow-up period neither the subjects' diet nor smoking could be controlled. It has been documented that both nutrition and tobacco smoking may change the levels of circulating nitrite and nitrites (1, 26). However,

the main conclusion of our study was drawn from observation of hospitalised subjects (in the first four weeks), when they had a similar hypolipemic diet, so the effect of this confounding factor was controlled. Thirdly, at the study start our patient subgroups differed in respect of the plasma NO metabolites concentration, which potentially might affect the final results. However, this difference was statistically insignificant (p=0.44). Besides, in opposition to the final results, at baseline the concentration of NO metabolites was lower in the relapsed alcoholics than in those who remained abstinent over the whole observation period. Obviously, our conclusion would be more reliable if the baseline levels of NO metabolites were identical in both groups. in a retrospective analysis of factors differing abstinent and relapsed subjects, so a strict patient selection was not possible then. Finally, CDT as an alcohol abuse marker was not determined in our study, but this test is still expensive and hardly available in Poland.

In conclusion, an increase or a slight decrease in the plasma NO metabolites concentration after four weeks' abstinence may predict relapse to alcohol drinking within the next five months. In view of some limitations of our study this problem requires further research.

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Address for correspondence:
Jacek Budzyński
Department of Gastroenterology,
Vascular Diseases and Internal Medicine,
The Ludwik Rydygier Medical University in Bydgoszcz,
75 Ujejskiego Street,
85-168 Bydgoszcz, Poland,
fax +48 52 371 49 12
e-mail: budz@cps.pl; budz@iweb.pl