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The dynamics of some oxidative stress markers in 3, 6 and 12-months alcohol abstinent patients: possible relevance for the usage of antioxidants in alcohol withdrawal

Dinamica unor markeri ai stresului oxidativ la 3, 6 şi 12 luni de abstinenţă de la alcool: posibila relevanţă a utilizării de antioxidanţi

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Abstract

While the exact relevance of the oxidative stress markers after the complex processes of alcohol withdrawal is still controversial, in the present report we were interested in studying the relevance of oxidative stress status in the alcohol withdrawal processes, by determining some oxidative stress markers after 3, 6 and 12 months of abstinence. 62 patients were selected, all of them males. Thus, 33 (baseline), 14 (3 months), 14 (6 months) and 15 (12 months) patients, while the control group (n=32) included healthy, sex and aged-matched subjects. Regarding superoxid dismutase, we observed a significant group difference ($p<0.0001$), together with an increase in all 3 cases of time-abstinence, as compared to baseline results: ($p<0.0001$-3 months), ($p<0.0001$-6 months) and ($p<0.0001$-12 months). Also for glutathione peroxidase, we observed a significant overall effect of the abstinence in our groups ($p=0.0003$), plus an increase especially at 6 months ($p=0.03$) and 12 months ($p=0.006$). Regarding malondialdehyde, as a main marker for the lipid peroxidation processes, we found significant differences between our groups ($p<0.0001$), together with a decrease in all 3 cases, compared to the baseline group ($p=0.003$), ($p=0.01$) and ($p=0.0002$). In conclusion, this confirms the increased oxidative stress status in alcoholic patients and even more importantly, we showed that there is a significant and progressive decrease in the oxidative stress status at 3, 6 and 12 months after the withdrawal process, as demonstrated by the increased levels of antioxidant enzymes and decreased rate of lipid peroxidation, when compared to baseline values.

***Key words**: alcohol; abstinence; oxidative stress.*

Rezumat

În prezentul articol am fost interesaţi să studiem relevanţa stresului oxidativ în cadrul proceselor legate de abstinenţa de la alcool, având în vedere în special faptul că literatura de specialitate este extrem de controversată

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în acest domeniu de cercetare. Astfel, am determinat nivelul unor markeri specifici ai stresului oxidativ la pacienţii selectaţi după 3, 6 şi 12 luni de abstinenţă de la alcool. 62 de pacienţi de sex masculin au fost selectaţi pentru studiu. În cadrul studiului s-au prezentat 33 de pacienţi pentru determinările bazale, 14 pacienţi la 3 luni, 14 pacienţi la 6 luni şi 15 pacienţi la 12 luni de abstinenţa de la alcool, în timp ce lotul control a inclus 32 de persoane sănătoase, potrivite ca vârstă si sex-ratio cu celelalte grupuri de studiu. Astfel, în ceea ce priveşte rezultatele, în cazul superoxid dismutazei (SOD) am observat o diferenţă semnificativă între cele trei loturi de studiu ($p<0.0001$), precum şi o creştere semnificativă din punct de vedere statistic a valorilor SOD la pacienţii aflaţi la 3 luni ($p<0.0001$), 6 luni ($p<0.0001$) şi respectiv 12 luni ($p<0.0001$) de abstinenţa de la alcool, faţă de determinările bazale. De asemenea, în cazul glutation peroxidazei, am observat o diferenţă semnificativă din punct de vedere statistic între grupuri ($p=0.0003$), plus creşteri importante la 6 luni ($p=0.03$) şi 12 luni ($p=0.006$), faţă de determinările bazale. În ceea ce priveşte malondialdehida (MDA), ca şi principal marker al proceselor de peroxidare lipidică, am putut observa de asemenea diferenţe semnificative între grupurile de studiu ($p<0.0001$). Mai mult, în cazul tuturor celor 3 grupe de pacienţi s-au putut observa scăderi semnificative ale concentraţiei de MDA, în comparaţie cu determinările bazale ($p=0.003$ pentru 3 luni, $p=0.01$ pentru 6 luni şi $p=0.0002$ pentru 12 luni). În concluzie, aceste date confirmă un stress oxidativ crescut la pacienţii consumatori cronici de alcool şi, mai important decât atât, demonstrează o scădere semnificativă şi progresivă a statusului stresului oxidativ la 3, 6 şi 12 luni de abstinenţa de la alcool, aşa cum am putut observa din creşterea progresivă a activităţii specifice a enzimelor antioxidante determinate şi scăderea nivelelor de peroxidare lipidică.

***Cuvinte cheie**: alcool; abstinenţă; stres oxidativ*

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Introduction

While it is generally accepted that there is an increased oxidative stress status in patients with alcohol dependence, mainly expressed through a reduction in the general antioxidant activity and a significant increase of the lipid peroxidation processes (1), the exact relevance of the oxidative stress markers after the complex processes of alcohol withdrawal is still controversial.

In this way, for all the markers of the oxidative stress, as in the case of the main antioxidant enzymes (superoxide dismutase-SOD and glutathione peroxidase-GPX), there are previous reports stating both increased and decreased activities ((2)-increased; (3)–decreased, for superoxide dismutase) ((4)-decreased; (5)-no modification at all, for glutathione peroxidase).

Also, the other side of the oxidative stress balance, which is represented by the reactive oxygen species, is reported to suffer controversial modifications during the process of abstinence. Thus, when talking about malondialdehyde-MDA as the main marker of the lipid peroxidation processes, previous reports described either increased levels in patients with alcohol withdrawal (6,7), as well as clear reductions in MDA levels following alcohol withdrawal (4,8).

Also, in our previous studies we showed a significant decrease of the oxidative stress status, one week and one month following the withdrawal, as demonstrated by a significant increase in the specific activity of SOD, as well as by a decrease in the MDA levels. However, as we showed back then, in the case of all three markers of oxidative stress status which we determined (SOD, GPX and MDA), the levels after one week or one month of abstinence were significantly altered when compared to controls (9).

Thus, in a continuation of our studies, in the present report we were interested in studying the importance of oxidative stress status in the alcohol withdrawal processes, by determining some oxidative stress markers after an even longer term: at 3, 6 and 12 months of abstinence and comparing them to the baseline and the control group.

Material and methods

62 patients were selected between January 2013- July 2014, aged between 26 to 79 years old (average 44.8 ± 3.7 years), all of them males. They met the Diagnostic and Statistical Manual of Mental Disorders IV Text Revision diagnostic criteria for alcohol dependence. Of course, alcohol consumption was stopped abruptly at admission.

Exclusion criteria were the following: illicit drug use, chronic systemic disease or severe mental disorders. All the patients were treated with diazepam or lorazepam.

In this way, 33 (at baseline), 14 (at 3 months), 14 (at 6 months) and 15 (at 12 months) patients from the initial 62 had their blood collected, since some of them decided to drop out of the study or refused blood collection at some point.

The control group (n=32) included healthy, sex and aged-matched subjects without any psychiatric or physical illnesses. Also, the controls did not meet the criteria for alcohol abuse/dependence or any abusive alcohol consumption in the last 2 months.

Blood samples were obtained in the morning, before breakfast; after being centrifuged, the serum was then put into plastic tubes and stored at −40°C until measurement. Determination of SOD, GPX and MDA were performed by using "19160 SOD" or "GPX CGP1" Cellular Activity Assay Commercial Kits or by using classical and well known methods (for MDA - 10).

The current study was performed under the approval of the Socola Hospital Ethics Committee. Also, signed consent was obtained from all patients, according to the World Medical Association Declaration of Helsinki, revised in 2000, Edinburgh.

Data Analysis

The levels of oxidative stress markers were statistically analyzed using one-way analysis of variance (ANOVA). All results are expressed as mean ± SEM. Post hoc analysis were then performed using Tukey's honestly significant difference test in order to compare all groups (except control) between them. F values for which $p<0.05$ were regarded as statistically significant.

Results

Regarding the superoxide dismutase results, we observed a significant group difference ($p<0.0001$) (Figure 1), suggesting significant effects of alcohol abstinence on SOD specific activity. Also, *post hoc* comparisons showed a significant increase in the specific activity in all 3 time-related abstinence cases, when compared to baseline results: ($p < 0.0001$ at 3 months), ($p < 0.0001$ at 6 months) and ($p < 0.0001$ at 12 months) (Figure 1). Still, there was a significant decrease in the SOD specific activity at 3 ($p < 0.0001$) and 6 ($p < 0.0001$) months, when compared to the control group. On the other hand, there were no significant modifications when we compared the control group with the 12 months group ($p = 0.99$) (Figure 1). In addition, we also observed a progressive increase in SOD's specif-

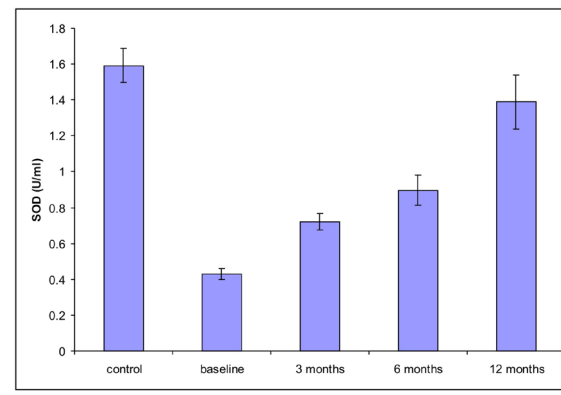

Figure 1. Superoxide dismutase (SOD) specific activity in the serum of control subjects, baseline and alcohol abstinent patients after 3, 6 and 12 months. The values are mean ± SEM (n = 32 in control group, n= 33 in baseline, n=14 in 3 months group, n=14 in 6 months group and n=15 in 12 months group).

ic activity, as the time from withdrawal increased, especially from 3 to 12 months (p =0.0003) and also from 6 to 12 months (p =0.009) (Figure 1).

When it comes to the results of the other antioxidant enzyme, which was GPX, we could also observe a significant overall effect of the abstinence on enzymatic specific activity in our groups (p=0.0003) (Figure 2). Moreover, when we performed the *post hoc* analysis, we observed a significant increase in the specific activity of the enzyme, especially at 6 months (p =0.03) and 12 months (p =0.006), compared to the baseline group (Figure 2). However, the specific activity at 3 months was still significantly decreased (p =0.026), when compared to the control group (Figure 2). Additionally, there was a progressive increase in the GPX specific activity from the time of withdrawal, as showed for example by the significant increase in the 6 months group, when compared to the 3 months group (p =0.007). Also, a significant difference was observed in the GPX specific activity between the 3 vs. 12 months group (p =0.001) (Figure 2).

Regarding the levels of malondialdehyde, as a main marker for the lipid peroxidation processes, we also found significant differences between our study groups (p<0.0001). In addition to that, when we performed the *post hoc* analysis, we observed a significant decrease for all the 3 cases we studied, when compared to the baseline group (p =0.003 at 3 months), (p = 0.01 at 6 months) and (p = 0.0002 at 12 months) (Figure 3). Still, no significant modifications were noticed when we compared our 3 study groups (p =0.07 at 3 months), (p = 0.19 at 6 months) and (p = 0.23 at 12 months) with the controls (Figure 3). Also, we observed a tendency for a progressive decrease of MDA in time, as showed for example by the significant decrease of the MDA levels in the 12 months group, as compared to the 6 months patients (p < 0.0001). Furthermore, a significant difference was observed in the GPX specific activity between the 3 vs. 12 months group (p =0.0001) (Figure 3).

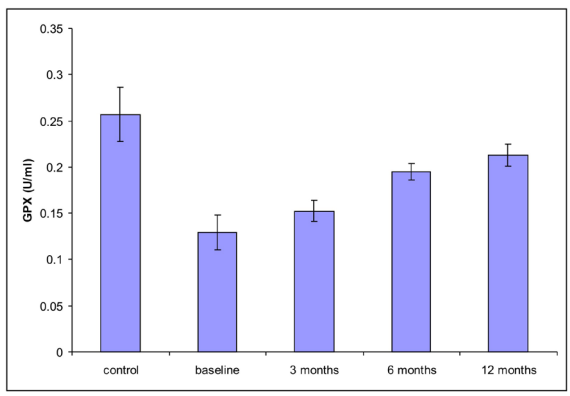

Figure 2. Glutathione peroxidase (GPX) specific activity in the serum of control subjects, baseline and alcohol abstinent patients after 3, 6 and 12 months. The values are mean ± SEM (n = 32 in control group, n= 33 in baseline, n=14 in 3 months group, n=14 in 6 months group and n=15 in 12 months group).

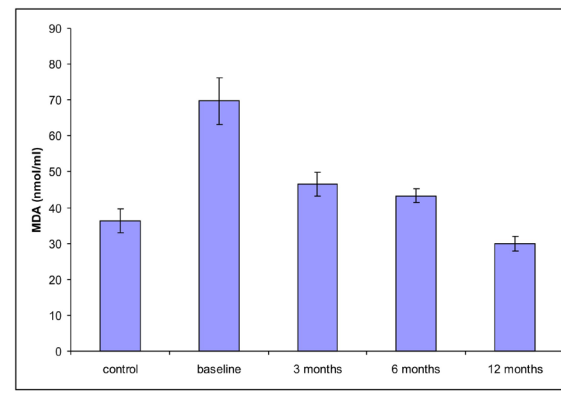

Figure 3. The levels of malondiadehyde (MDA) in the serum of control subjects, baseline and alcohol abstinent patients after 3, 6 and 12 months. The values are mean ± SEM (n = 32 in control group, n= 33 in baseline, n=14 in 3 months group, n=14 in 6 months group and n=15 in 12 months group).

Discussion

In this way, the data we presented in this study confirmed again the increased oxidative stress status in alcoholic patients and even more importantly, we showed that there is a significant and progressive decrease in the oxidative stress status at 3, 6 and 12 months after the withdrawal process, as demonstrated by the increased levels of antioxidant enzymes and decreased rate of lipid peroxidation, when compared to baseline values.

This data are also an important continuation of our previous studies, in which we demonstrated a decrease in the oxidative stress status, one week and one month following the withdrawal, as showed by a significant increase in the specific activity of SOD, as well as by a decrease in MDA levels, when compared to baseline. Still, in the case of all three markers of the oxidative stress status which we determined back then, the levels from one week or one month of abstinence were significantly altered when compared to controls, suggesting that severe and prolonged deficiency in their levels needs longer than one month of abstinence to normalize (9).

All these aspects could lead to the idea of using antioxidant compounds in order to reduce or improve the damages produced by alcohol consumption/withdrawal. In this way, it was showed for example that procysteine, which is a glutathione precursor (11), could increase the alcohol-depleted glutathione stores in various muscles of a rat model, folowing a period of abstinence, especially since it is known that alcohol consumption may result in numerous negative muscular effects (12). Thus, glutathione restoration therapy could provide therapeutic benefits to the overall antioxidant state of skeletal muscles, especially when it is used in conjunction with an established detoxification program for the recovering alcoholics, as Otis et al. suggested (12).

Another important antioxidant drug in this area of research is represented by N-acetylcysteine, which was experimentally used, for example, for the myocardial oxidative stress in alcoholic heart disease (13). Also, it seems that alcohol related oxidative stress could be in fact inhibited by N-acetylcysteine (14). Moreover, there seems to be an interaction between N-acetylcysteine's metabolism and the withdrawal processes, which could result in decreased oxidative stress levels (15). Importantly, as in the case of the procysteine, the protective effects of N-acetylcysteine could also be explained by the fact that it is required for glutathione biosynthesis (16), which is of course an important antioxidant, with fundamental roles in preventing damages induced to important cellular components by the free radicals and various peroxides (17).

Thus, drugs like glutathione, procysteine and NAC are right now in our attention for their possible therapeutic actions in the withdrawal processes, both in animal models, as well as for human patient studies.

In this way, we generally demonstrated the fact that a decrease of the oxidative stress level is sustained by all measured parameters both on short term (9) and long term, as demonstrated through the results of the present report.

Thus, the metabolism of the oxidative stress could be a fundamental aspect in the mechanistic of withdrawal and perhaps it may represent a central point where other negative factors are meeting, resulting in this complicated set of events. However, there is a long way until we can establish a clear relationship between antioxidant-related deficiencies and alcohol consumption/withdrawal time, especially considering the importance of free radicals in many metabolic reactions, but also due to the fact that in the present paper we actually showed a natural evolution for the oxidative stress status.

Regarding the limitations of the present study, we could add the fact that all the groups

received B vitamin supplements that could have influenced our results (however all the subjects received the same combination of B1+B6), also the lack of calculation power for this study, in order to see the number of subjects included (we rather used the patients which met the inclusion and exclusion criteria through the mentioned duration of the study, while trying to have a large-enough group of subjects and controls), but also a more strict diet, body mass index and alcohol quantity determinations before withdrawal.

Conclusions

In conclusion, our results are suggesting that there is a significant and progressive decrease in the oxidative stress status at 3, 6 and 12 months after the withdrawal process, as demonstrated by the increased levels of antioxidant enzymes and decreased rate of lipid peroxidation, when compared to baseline values. This could be relevant for the beneficial and therapeutical actions of the antioxidants usage in the withdrawal processes.

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