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Similar effects of chronic voluntary alcohol intake and high dose superoxide dismutase gene delivery on oxidative and carbonyl stress in rats

Research Article

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Summary

Experimental and clinical studies demonstrate a crucial role of alcohol in the etiology of various diseases. Oxidative stress is among the major mechanisms of detrimental effects of alcohol. Our previous study showed a protective effect of low molecular weight antioxidants on the level of oxidative and carbonyl stress markers in a model of chronic compulsory alcohol intake. The aim of this study was to evaluate the effects of an enzymatic high molecular weight antioxidant (manganese superoxide dismutase - Mn-SOD) gene delivery on oxidative and carbonyl stress in a rat model of chronic voluntary alcohol intake. Male Wistar rats received either water (control, SOD) or alcohol solutions (alcohol, alcohol + SOD) instead of drinking water during a period of 28 days. The concentration of ethanol gradually increased every week (5, 10, 15, 20% v/v). Plasmid encoding mitochondrial Mn-SOD or saline was injected intramuscularly once a week. At the end of the study, the levels of advanced glycation end products (AGEs) and ascorbic free radicals in serum were decreased by both, chronic alcohol treatment and SOD gene application independently. These effects had an additive potential, as the combination of alcohol and SOD treatment resulted in the lowest levels of these markers ($p<0.001$). As expected, SOD gene therapy decreased the levels of serum advanced oxidation protein products (AOPP). Interestingly, chronic alcohol intake had no effect on the oxidative damage of proteins and the combination of treatments significantly increased AOPP ($p<0.001$). Further, alcohol and SOD independently decreased lipoperoxidation in cerebellum, as estimated by malondialdehyde measurement ($p<0.05$). Malondialdehydelevels in alcohol + SOD group were similar to those observed in the control group. In summary, carbonyl and oxidative stress markers were decreased by a high dose SOD gene therapy as well as by chronic voluntary alcohol intake. The combination treatment resulted in contradictory effects on the monitored parameters – additive, if considering antioxidative status and AGEs production; antagonistic, if considering malondialdehydeand AOPP levels. Further studies are needed to explain and prove these results.

I. Introduction

Ethanol is among many chemical substances that have been the subject of man's interest for ages. Experimental and clinical studies demonstrate a crucial role of alcohol in the etiology of various diseases (Bau et al., 2007; Parry et al., 2011; Wang et al., 2010). However, despite extensive detrimental impacts of alcohol on human health, there is also a bright side that includes for example the so-called "French paradox"(Lippi et al., 2010; Sun et al., 2001). This phenomenon refers to a markedly decreased incidence of cardiovascular diseases in France compared to other European countries with similar nutrition habits, which is in general ascribed to higher red wine consumption likely from cultural reasons. The red wine compounds that are possibly responsible for this observation include procyanidins, polyphenols, but also alcohol. Such a bidirectional action of alcohol indicates the need for extensive research in this field.

Oxidative stress is considered one of the main mechanisms of toxic ethanol effects (Cederbaum et al., 2009; Ha et al., 2010). Oxidative stress is defined as a dysbalance between the increased production of free radicals and the antioxidative status of cells. Free radicals are mostly oxygen-derived reactive molecules called reactive oxygen species (ROS). These reactive molecules play an important role in physiological processes such as signal transduction or immune response (Finkel, 2011). Alcohol has been known for inducing oxidative stress, although the detailed pathomechanism remains unknown. Free aldehyde groups of the ethanol metabolite acetaldehyde can interfere with amino acids and change protein structure to form new epitopes that activate autoimmune processes. The possible sources of pathological ROS production involve increased activity of cytochrome P450 2E1 (CYP2E1), increased level of free iron ions and activation of immune responses (Cederbaum et al., 2009). However, it is not completely clear, if these processes are the cause or the consequence of alcohol induced tissue damage. ROS act as damaging agents on various levels, including protein impairment, lipoperoxidation and DNA mutations.

Most of the cell types possess a number of protective mechanisms against oxidative stress. The major players in antioxidative processes are antioxidative enzymes, which involve catalase, glutathione peroxidase/reductase and superoxide dismutase (SOD). SOD is one of the best described antioxidative enzymes that catalyses the dismutation of superoxide radicals. Previous study showed the protective effect of low molecular weight antioxidants (Vitamin C + Vitamin E in combination) on the level of oxidative and carbonyl stress markers in a model of chronic compulsory alcohol intake via gastric gavage (Celec et al., 2003). The aim of this study was to evaluate the effects of an enzymatic high molecular weight antioxidant (manganese SOD - Mn-SOD) gene delivery on oxidative and carbonyl stress in a model of chronic voluntary alcohol intake.

II. Materials and Methods

2.1 Experimental procedure

Male Wistar rats (n = 27; 250-280 g) were randomly divided into four groups: Control (CTRL; n = 6), Alcohol (ALC, n = 7), SOD (n = 7), Alcohol + SOD (ALC + SOD; n = 7). Rats were fed *ad libitum* standard chow and received either water (CTRL, SOD) or alcohol solutions (ALC, ALC + SOD) instead of drinking water during a period of 28 days. The concentration of ethanol gradually increased every week (5, 10, 15, 20% v/v). Alcohol consumption was monitored daily during the whole study. Plasmid pcDNA3 containing the gene encoding mitochondrial Mn-SOD (300 μg in 100 μl saline; SOD, ALC + SOD) or the same volume of saline (CTRL, ALC) was administered intramuscularly into the musculus biceps femoris of the left hind limb once a week. At the end of the study rats were sacrificed by decapitation in anesthesia. Blood and cerebellum samples were collected. Serum and tissue homogenates were used for biochemical analyses. Tissue homogenates were prepared in phosphate buffer saline to obtain 30% w/v homogenates. The study protocol was approved by Local Ethics Committee. The research was conducted according to the NIH Guideforthe Care and Use ofLaboratoryAnimals.

2.2 Carbonyl stress parameters

Serum samples were diluted 1:50 to measure the advanced glycation end products (AGE) - specific autofluorescence at the excitation wavelength of 350 nm and emission wavelength 450 nm (Munch et al., 1997).

2.3 Oxidative stress parameters

Malondialdehyde (MDA) was measured spectrophotometrically in cerebellum at 532 nm using the reaction with thiobarbituric acid as described previously (Torres et al., 2004). Serum advanced oxidation protein products (AOPP) were measured spectrophotometrically (340 nm) according to a previously described method using chloramin T and potassium iodide as a standard (Witko-Sarsat et al., 1998).

2.4 Antioxidant capacity

Electron paramagnetic resonance (EPR) method using 5,5-dimethyl-1-pyrroline N-oxide (DMPO) as a spin trapping agent and nitroxide radical 4-hydroxy-2,2,6,6-tetramethylpiperidine N-oxyl (TEMPOL) as a standard was performed to evaluate the antioxidative status of serum samples (Reinke, 2002). Samples were irradiated with an ultraviolet lamp (300-400 nm; 30 mW cm-2) and the concentrations of free ascorbic radical were estimated before and after 1 and 5 min of irradiation.

2.5 Statistical analysis

Statistical analyses were performed using XLStatistics 10.05.30 (XLent Works, Australia). Differences between groups were tested using One-way ANOVA and Scheffé post hoc test. P-values less than 0.05 were considered significant. The symbols express significant difference against CTRL group. * - $p<0.05$; *** - $p<0.001$.

III. Results

The consumption of alcohol and water in the respective groups did not differ significantly throughout the experiment. Average water intake was 29 and 28 ml/rat/day, respectively, in CTRL and SOD groups. Average alcohol intake was 32 ml/rat/day in ALC and ALC + SOD groups and did not vary as the concentration was increasing. As shown on Figure 1, AGE-specific fluorescence of serum was decreased by both, chronic alcohol treatment and SOD gene application.

These effects had an additive potential as the combination of alcohol and SOD treatment resulted in the lowest levels of these markers of carbonyl stress ($p<0.001$). As expected, SOD gene therapy decreased the levels of serum AOPP. Interestingly, chronic alcohol intake had no effect on the oxidative damage of proteins and the combination of treatments significantly increased AOPP (Figure 2; $p<0.001$). Further, alcohol and SOD independently decreased lipoperoxidation in cerebellum, as estimated by MDA measurement (Figure 3; $p<0.05$). MDA levels in ALC + SOD group were similar to those observed in the control group. Finally, one minute of UV-irradiation of serum samples produced most ascorbic free radicals (AFR) in the control group. All treatment groups showed lower AFR production, which indicates a higher antioxidative status (Figure 4; $p<0.05$). Similarly to AGEs, the lowest level of AFR was observed in ALC + SOD group.

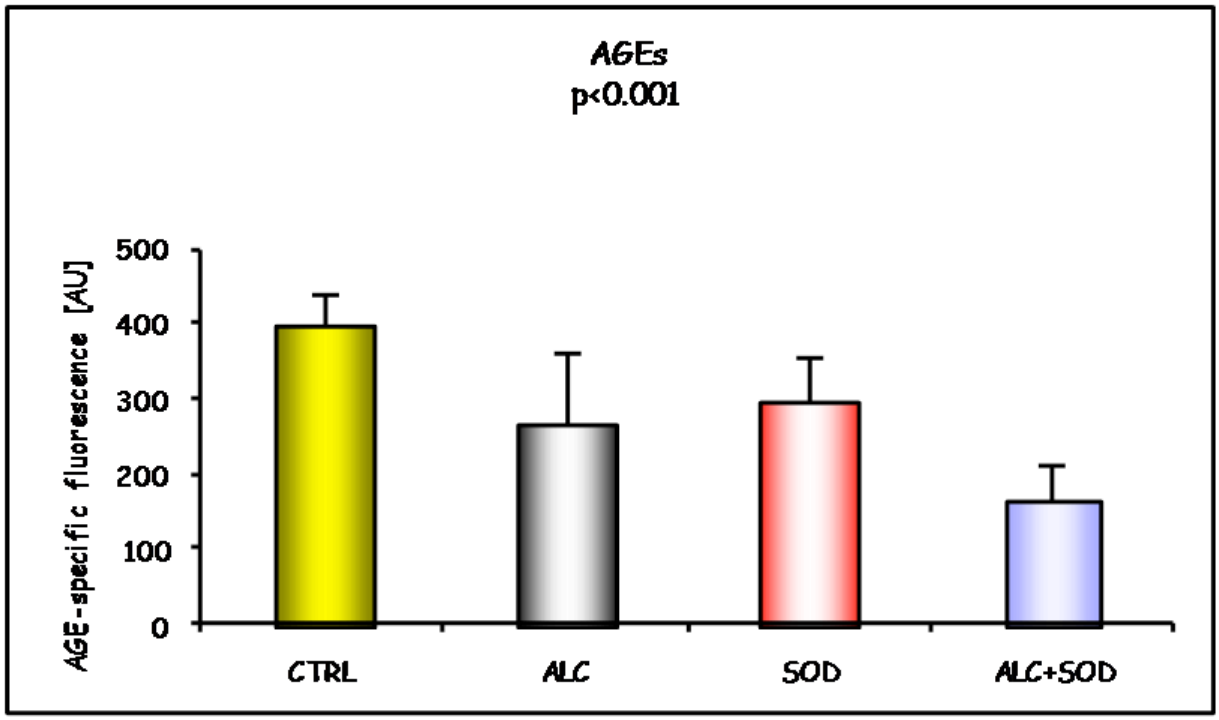

Figure 1: Effect of alcohol consumption and superoxide dismutase gene delivery on advanced glycation end products (AGEs) in serum.

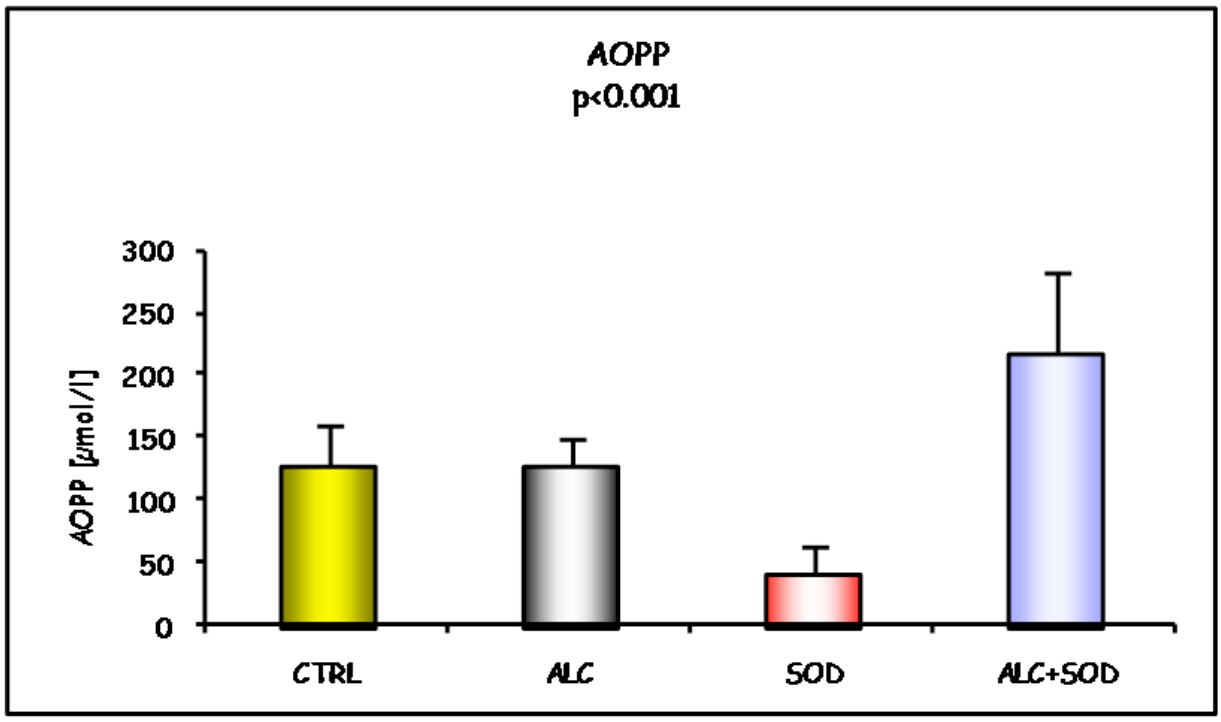

Figure 2: Effect of alcohol consumption and superoxide dismutase gene delivery on advanced oxidation protein products (AOPP) in serum.

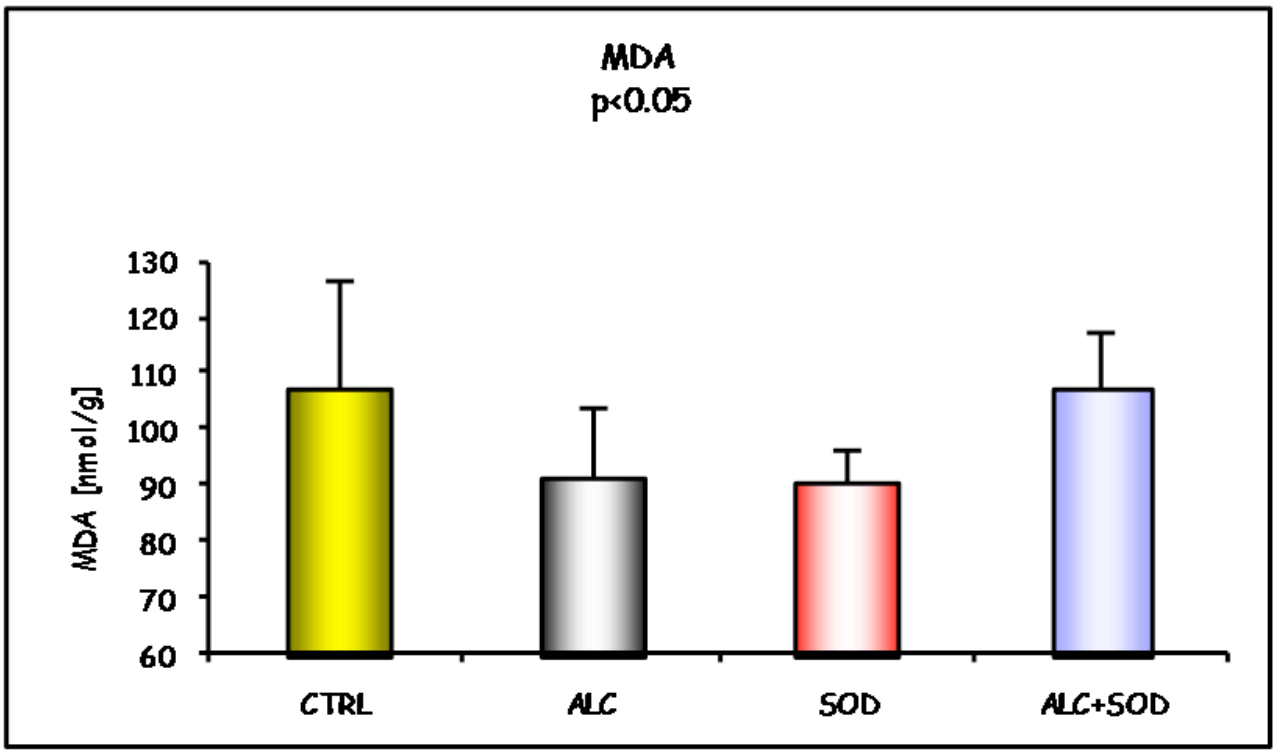

Figure 3: Effect of alcohol consumption and superoxide dismutase gene delivery on malondialdehyde in cerebellum.

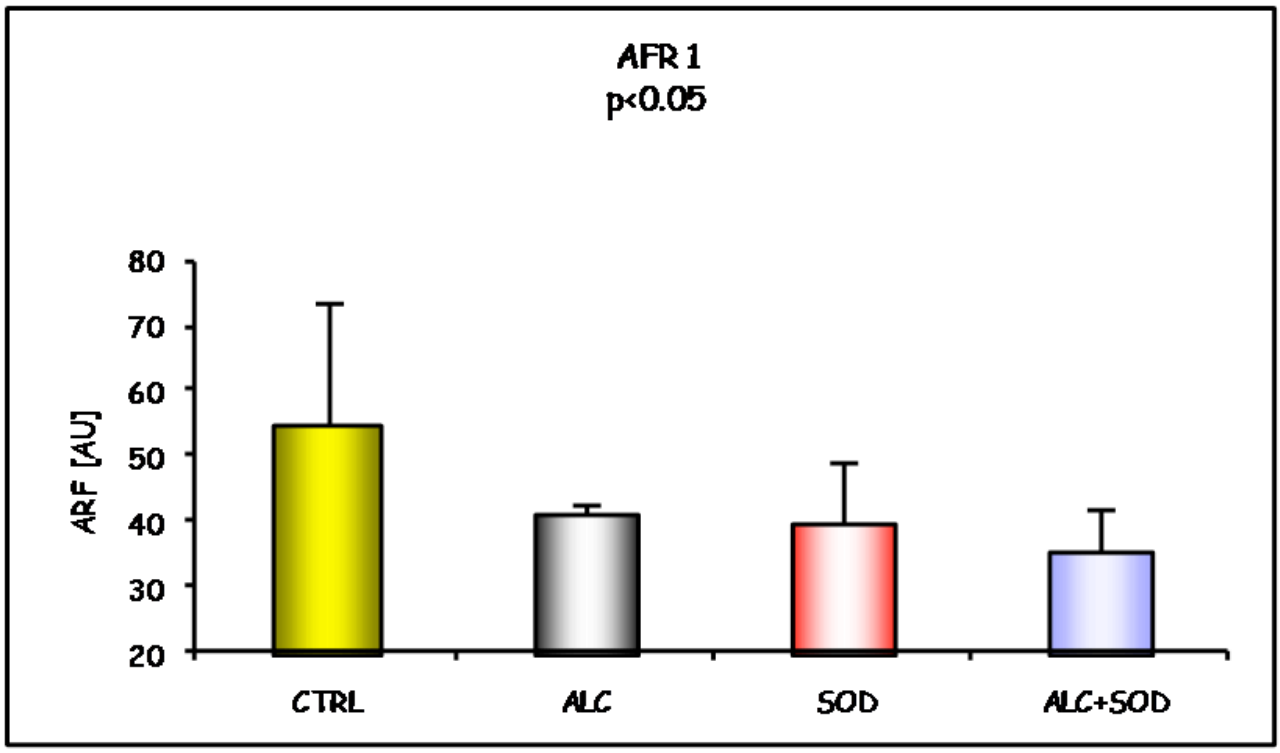

Figure 4: Effect of alcohol consumption and superoxide dismutase gene delivery on ascorbic free radicals in serum.

IV. Discussion

Our surprising results indicate that alcohol decreased carbonyl and partially even oxidative stress. AGE-specific fluorescence of serum was decreased by both, chronic alcohol treatment and SOD gene application. The combination of alcohol and SOD treatment resulted in the lowest levels of this parameter of carbonyl stress, indicating an additive effect. This finding supports the hypothesis that the protective effect of red wine could be mediated via the combination of specific dose of alcohol and antioxidants. One of the molecular pathways that might explain the paradox of beneficial alcohol intake is the cardioprotective effect of acetaldehyde – an intermediate in alcohol metabolism that clearly decreases carbonyl stress parameters, as described in a well-known study by Al Abed and colleagues (Al-Abed et al., 1999). Oxidative and carbonyl stress interdependently cooperate when it comes to tissue damage. Inhibiting one kind of stress likely stops both of them.

AOPP are the markers of oxidative damage of proteins. As expected, SOD gene therapy decreased the levels of serum AOPP. Interestingly, chronic alcohol intake had no effect on the oxidative damage of proteins and the combination of treatments significantly increased AOPP. The results in ALC + SOD group indicate a possible antagonistic effect that might be explained by the inhibitory effect of ethanol on catalase activity that results in decreased hydrogen peroxide degradation, while its production is increased by applied SOD (Noh et al., 2011). In the light of the results in other parameters, it is surprising that alcohol does not decrease AOPP. We hypothesize that the decrease of oxidative and carbonyl stress induced by alcohol treatment cannot be translated into decreased AOPP levels because of the catabolic effect of ethanol on proteins and their turnover. The reduced turnover of proteins may antagonize the antioxidative effects via increased half time of protein degradation.

Cerebellum is the organ that is most vulnerable to oxidative stress (Luo, 2012). Alcohol and SOD independently decreased lipoperoxidation in cerebellum, as shown by MDA measurement. MDA levels in ALC + SOD group are similar to those observed in the control group. The previously mentioned mechanism via catalase inhibition might also explain these findings.

EPR is a highly specific method, as it detects free radical production directly, not only markers of oxidative stress (Reinke, 2002). One minute of UV-irradiation of serum samples produced the highest amount of AFR in the control group. All treatment groups showed lower AFR production, which indicates a higher antioxidative status in these groups. Similarly to AGEs results, an additive effect of alcohol and SOD was found in this parameter.

In the previous study performed by our group, AGEs level in serum was increased in ethanol fed group (Celec et al., 2003). Alcohol was administered via gastric gavage in a fixed concentration (40% v/v) during

the whole experiment. On contrary, the current study showed opposite results demonstrated by decreased level of AGEs in a model of voluntary alcohol intake. This indicates that the way of ethanol administration as well as ethanol concentrations might play a key role in the relationship between alcohol and carbonyl stress. Further, the current study showed that the effects of chronic voluntary alcohol intake and high dose SOD gene delivery are similar, at least from the view of decreased levels of some oxidative and carbonyl stress markers. However, the hypothesis on similar effects of alcohol and SOD gene delivery is not supported by AOPP measurement results, which show an antagonistic effect of the combined treatment. As mentioned in the second paragraph, this may be caused by higher hydrogen peroxide level, which results from its high production rate and low degradation (high SOD expression and low catalase activity).

The administration of naked plasmid DNA is generally characterized by low transfection efficiency. This limitation can be compensated by the presence of a strong cytomegalovirus (CMV) promoter and a high dose of applied plasmid DNA (Gardlik et al., 2005). It is known that after plasmid injection into the muscle, the transgene is being expressed and the product secreted into the bloodstream (Maruyama et al., 2000). This provides a rationale for intramuscular DNA delivery and measurement of the oxidative stress parameters in serum described in the current study.

Results from the current experiment are partially in line with a different study, in which a lack of harmful effects was seen after 30 days of alcohol consumption designed to mimic the mediterranean pattern of alcohol intake (Martin et al., 2011). However, the ethanol concentration was as low as 1% in that study and, therefore, the comparison might not be relevant. In addition, available data on ethanol consumption so far justify the conclusion with regard to cardiovascular risk that the pattern of drinking is of more importance than the content of the bottle (van de Wiel and de Lange, 2008). This, together with the fact that the rodent ethanol metabolism differs from the human makes it difficult to compare data from the literature and to draw any definitive conclusion applicable to human settings.

In summary, carbonyl and oxidative stress markers were decreased by a high dose SOD gene therapy as well as by chronic voluntary alcohol intake. The combination treatment resulted in contradictory effects on the monitored parameters – additive, if considering antioxidative status and AGEs production; antagonistic, if considering malondialdehyde and AOPP levels. Further studies are needed to explain and prove these results.

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