Oxidative damage in young alcohol drinkers: A preliminary study

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Abstract
Background: Oxidative damage (OD) biomarkers have been used to evaluate metabolic stress undergone by alcoholic individuals. In alcoholic patients, these biomarkers are usually measured at late stages, i.e., when the alcoholic patients are showing clear signs of impaired hepatic function. OD biomarkers are sensitive indicators of impaired metabolic function, and might be useful in early stages of alcohol consumption to identify individuals who are at greater risk of damage in later stages of alcohol consumption. The aim of the present work was to evaluate some OD biomarkers in young people at early stages of alcohol consumption.

Methods: The study was carried out in a group of young people (18–23 years old) who drank alcohol, Youngsters Exposed to Alcohol (YEA) with an average intake of 118 g of ethanol/week, and a control group (CG) of non-drinkers. Blood counts, alcohol dehydrogenase (ADH) activity, glutathione peroxidase (GSH-Px) activity, oxidative damage to DNA, and lipid peroxidation were determined in both groups.

Results: The anthropometric and blood parameters of both groups were similar and no clinical symptoms of hepatic damage were observed. Nevertheless, ADH activity, lipid peroxidation, and percentage of damaged DNA cells were higher in the YEA group than in the control group. In contrast, GSH-Px activity was lower in the YEA group than in the control group.

Conclusion: Alteration in OD biomarkers can be found in individuals with 4–5 years of alcohol drinking history. To our knowledge, this is the first study giving evidence of OD in individuals at early stages of alcohol abuse.

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Introduction
Alcohol use is the third-highest risk factor for disease or injury in developed countries, and the greatest risk factor in developing countries with low child and low adult mortality, as classified by WHO (WHO, 2002). The excessive intake (either acutely or chronically) of alcohol induces physical and mental illness. Most studies on alcohol biomarkers include participants with evident chronic effects of alcohol abuse such as liver inflammation, gastritis, delirium tremens, or behavioral disorders (Brien, Ronksley, Turner, Mukamal, & Ghali, 2011; Hingson, Heeren, Winter, & Wechsler, 2005). Participants in these studies had been using alcohol for more than 10 years. Studies of acute toxicity of alcohol include individuals with a wide range of ages (Frias, Torres, Rodriguez, Ruiz, & Ortega, 2000; Rauchenzauner et al., 2005). Several biomarkers and symptoms are evaluated, such as blood alcohol concentration, blood pressure, academic performance, and behavioral disorders, among others. It is assumed that those individuals showing effects from acute alcohol toxicity will later show more severe effects from chronic alcohol toxicity. Nevertheless, the mechanisms and target organs of acute alcohol toxicity differ from chronic alcohol toxicity. The acute effects are seen in the central nervous system, while chronic effects are mediated by oxidative damage in the liver (Castañeda, 2009). In understanding these differences, some questions arise: How long does an individual have to be exposed to alcohol to show detectable chronic effects? At which stage is it possible to know if an individual will exhibit effects from chronic alcohol exposure? Could a biomarker be detected in early stages before greater damage is caused by chronic toxicity?
Although the precise mechanisms of chronic ethanol toxicity are still unclear, evidence in the last decade from studies in animal models or human populations has pointed out the importance of OD in the pathophysiological complications of chronic alcohol use (Haorah et al., 2008). OD results in the oxidation of biomolecules such as lipids or DNA, whose function could be severely impaired (Frias et al., 2000). Therefore, lipid peroxidation (LPO) and DNA damage are often used as OD biomarkers (Huang, Chen, Peng, Tang, & Chen, 2009; Peng et al., 2005; Shanmugam, Ramakrishna, Mallikarjuna, & Reddy, 2009; Yang et al., 2010).

Studies on chronic effects of alcohol on human populations have been conducted in patients with several years of alcohol intake, i.e., when clinical signs of impaired hepatic function are evident. However, an obvious choice for biomarkers in early stages of alcohol toxicity are the OD-sensitive biomarkers, since OD is part of the mechanism of chronic toxicity. So, LPO would be an excellent biomarker for early stages of chronic alcohol toxicity, i.e. within a time interval before greater damage is observed. The aim of this work was to study oxidative damage in young people (18–23 years old) who have been abusing alcohol for less than 5 years and do not show hepatic disorders. In the present paper, we describe preliminary results on several biomarkers measured in young people with a relatively short history of alcohol consumption.

Materials and methods

Reagents

All reagents were purchased from Sigma Chemical Company (St. Louis, MO) except EDTA and menadione which were obtained from Merck, USA. All substances were of analytical grade.

Sampling and OD parameters

A cross-sectional comparative study was carried out between the 2 groups: the YEA group and non-drinking (self-reported) control individuals (CG). The inclusion criteria were: young people (18–23 years old) who have been abusing alcohol for less than 5 years and do not show hepatic disorders. In the present paper, we describe preliminary results on several biomarkers measured in young people with a relatively short history of alcohol consumption.

Results

The YEA and control groups appeared to be clinically healthy and without behavioral disorders. There were no differences in anthropomorphic or hematological parameters between groups (Table 1). The control group (CG) reported no alcohol drinking and the subjects in the YEA group had begun alcohol consumption no more than 5 years before the start of the study. On average, individuals in the YEA group drank 118 g of alcohol/week. The average value of ADH activity for the YEA group (3.57 ± 0.73 U/L) was 92% higher than for the CG group (1.85 ± 0.23 U/L; p = 0.0002). The YEA group showed oxidative damage as evaluated by comet assay and lipid peroxidation. Forty-four percent of the cells from the YEA group and 8% of the cells from the CG had tails. Tail length never exceeded 10 nm in any case. This result suggests that the YEA group had incipient DNA damage. Likewise, the YEA group showed higher LPO (2.04 ± 0.26 μmol/L) than CG (1.03 ± 0.39 μmol/L; p = 0.0003). An opposite effect was observed in GSH-Px activity. The YEA group had slightly lower GSH-Px activity (10.18 ± 1.77 U/g Hb) than CG (12.27 ± 1.13 U/g Hb; p = 0.0046).

Discussion

The drinking habits of the YEA group did not create social, work, or academic problems. The reasons for the drinking behavior among the YEA group are beyond the aim of the present study. The ADH increase may indicate an induction of the enzyme which could up-regulate by alcohol (Haseba & Ohno, 2010). In addition to this, other substances (TBARS) (Jain, McVie, Duett, & Herbst, 1989). A molar extinction coefficient of 35 mM−1 cm−1 of NDMA was used for calculations.

Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CG</th>
<th>YEA</th>
<th>Normal values</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>19.6 ± 1.1</td>
<td>19.8 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.48 ± 8.5</td>
<td>74.95 ± 22.6</td>
<td></td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>113 ± 2.6</td>
<td>116 ± 4</td>
<td>90–140</td>
</tr>
<tr>
<td>Systolic</td>
<td>71 ± 2.3</td>
<td>71 ± 2.3</td>
<td>70–90</td>
</tr>
<tr>
<td>Diastolic</td>
<td>17.65 ± 0.81</td>
<td>17.82 ± 0.4</td>
<td>12–20</td>
</tr>
<tr>
<td>Respiratory frequency (min−1)</td>
<td>None</td>
<td>118 ± 0.022</td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption (g alcohol/week)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (mg/dL)</td>
<td>19.7 ± 0.6</td>
<td>20.5 ± 0.5</td>
<td>15–20</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>53.6 ± 1.3</td>
<td>52.2 ± 0.7</td>
<td>40–55</td>
</tr>
<tr>
<td>Erythrocyte (10^6 cells/mm³)</td>
<td>5.2 ± 0.2</td>
<td>4.7 ± 0.2</td>
<td>4.0–6.6</td>
</tr>
<tr>
<td>Leukocytes (10^6 cells/mm³)</td>
<td>7405 ± 290</td>
<td>6213 ± 424</td>
<td>5000–11,000</td>
</tr>
<tr>
<td>Platelets (10^9 cells/mm³)</td>
<td>292 ± 2.5</td>
<td>212 ± 1.9</td>
<td>150–450</td>
</tr>
<tr>
<td>ADH activity (UL)</td>
<td>1.85 ± 0.23</td>
<td>3.57 ± 0.73*</td>
<td></td>
</tr>
<tr>
<td>TBARS (μmol/L)</td>
<td>1.03 ± 0.39</td>
<td>2.04 ± 0.26*</td>
<td></td>
</tr>
<tr>
<td>Damaged DNA from comet assay (% of counted cells with tail)</td>
<td>8%</td>
<td>44% (odds ratio: 8.897)</td>
<td></td>
</tr>
<tr>
<td>GSH-Px (U/g Hb)</td>
<td>12.27 ± 1.13</td>
<td>10.18 ± 1.77*</td>
<td></td>
</tr>
</tbody>
</table>
ethanol metabolism itself alters the cell redox state and can produce reactive oxygen species that may play an important role in the noxious effects of alcohol via oxidative stress (Haorah et al., 2008).

The results demonstrate that the YEA group has oxidative stress. Since GSH-Px is involved in the antioxidant mechanism, some antioxidant responses could be inferred from changes in its levels (Haleng, Pincemail, Defraigne, Charlier, & Chapelle, 2007). Nevertheless, the effect on GSH-Px could be organism-, organ-, and time-of-exposure-dependent. For instance, in the fish Jenynsia multidentata, GSH-Px is increased in brain and gills but decreased in liver and muscle after an exposure to an oxidant agent (Ballesteros, Wunderlin, & Bistoni, 2009). In humans, Rajdl, Racek, Trefil, and Siala (2007) observed an initial weak increase in GSH-Px after white wine consumption, but after a month of drinking white wine, GSH-Px levels returned to normal, revealing an adaptation to oxidative stress. This contrasts with our results, and could be explained by differences in age (42 years versus 19 years), genetics, and the likely duration of exposure to alcohol. Nevertheless, our results are congruent with those reported by both Uçar, Demir, and Ulug (2005) and Huang et al. (2009), who described groups chronically exposed to alcohol that had decreased GSH-Px activity. In the latter work, the individuals had an average of 13.2 years of alcohol dependence and the average age was 42 years. In the present work, participants have fewer years of alcohol intake and a lower age. In spite of these differences, a similar effect on GSH-Px activity was observed. Possibly, less than 5 years of alcohol exposure are sufficient to induce a decrease in GSH-Px activity.

Whatever the regulatory mechanism is, our GSH-Px results could be explained as a part of the antioxidant response. As a part of the cell response, GSH-Px activity is increased when an organism is under oxidative stress (Dlugosz, Pruss, & Lembas-Bogaczyk, 2010; Tseng et al., 2008). Thus, we can expect a positive correlation between OD biomarkers and GSH-Px. In the present work, the LPO biomarker TBARS correlates with GSH-Px activity ($r^2 = 0.3529$).

There are other mechanisms by which cells could avoid oxidative stress. In the case of alcoholism, oxidative stress is not induced by ethanol itself. Rather, the oxidative stress results when ethanol is metabolized by enzymes other than ADH. When ADH activity is able to efficiently metabolize ethanol, a lower level of oxidative damage might be expected, and hence a lower GSH-Px level. Thus, an inverse correlation between ADH activity and GSH-Px activity is to be expected. Indeed, in our work, higher GSH-Px activity correlates with lower ADH activity ($r^2 = 0.4030$). This is in agreement with reports on ADH2 knockout mice which have slightly increased GPx activity (Sang-Yong et al., 2007). As mentioned above, previous studies on humans have shown that GSH-Px is down-regulated by alcohol in groups with several years of alcohol intake: different types of damage can result, such as lower red blood cell count or liver damage. In the present work, the YE group — with few years of alcohol intake — showed a similar effect on GSH-Px, but no clinical differences in red blood count or any other hematological parameter were found, compared to controls. Further studies are required to evaluate the effect of the different ADH isoforms on alcohol-induced oxidative damage. To our knowledge, this is the first work indicating that oxidative damage could be observed at early stages of alcohol dependence. The oxidative damage in young subjects exposed to alcohol is evident; this could be used to evaluate effects of alcohol and prospective risks for young people. Before using oxidative biomarkers to diagnose and evaluate early alcohol abuse, further studies are needed to (1) identify additional biomarkers, (2) determine the dependence of OD biomarkers on duration of ethanol exposure, and (3) determine the correlation of OD biomarkers with other biomarkers of ethanol susceptibility (such as polymorphisms).

### Author disclosures

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Funding for this study was provided by CONACYT, who had no further role in study design, data collection, analysis or interpretation.

#### Contributors

Rendón-Ramírez Adela and Cortés-Couto Miriam designed and carried out the experiments. Martínez-Rizo Abril Bernardette, Muñiz-Hernández Saé and Velázquez-Fernández Jesús Bernardino along with Rendón-Ramírez Adela discussed and analyzed results, also they undertook the statistical analysis. All authors contributed to and have approved the final manuscript.

#### Conflict of interest

All authors declare that they have no conflicts of interest.

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### References


