THE EFFECT OF LOW CONCENTRATIONS OF ETHANOL ON GASTRIC ADENOCARCINOMA CELL LINES

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Abstract - Chronic alcohol consumption was identified as a significant risk factor for cancer in humans. The aim of the study was to analyze the influence of low concentrations of ethanol on gastric adenocarcinoma cell viability, apoptosis, and changes in the expression of alcohol dehydrogenase with ethanol treatment. Gastric adenocarcinoma cell lines (MGC803, MGC823 and SGC7901) were treated with different concentrations of ethanol (0.03125%, 0.0625%, 0.125%, 0.25%, 0.5%, 1%, 2%, and 4%). The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and flow cytometry were used to analyze the effect of ethanol treatment on cell viability and apoptosis. Western blotting was used to analyze the expression of alcohol dehydrogenase in gastric carcinoma cells. Ethanol treatment inhibited cell proliferation in gastric adenocarcinoma cell lines in a significant dose-dependent manner. Ethanol induced apoptosis of gastric adenocarcinoma cells in a dose-dependent manner. The alcohol dehydrogenase activity of gastric adenocarcinoma cells increased with the increase in the concentration of ethanol. Ethanol inhibited cell viability and the growth of gastric adenocarcinoma cell lines. Low concentrations of ethanol also induced apoptosis and increased the expression of alcohol dehydrogenase of the gastric adenocarcinoma cell lines.

Key words: Ethanol, gastric adenocarcinoma, apoptosis, alcohol dehydrogenase

INTRODUCTION

Alcohol is the oldest and most widely used drug in the world. There are possible health benefits in the consumption of low levels of alcohol, while effects can be severely detrimental in cases of chronic alcohol abuse. Heavy drinking over a long period of time can lead to alcoholic liver disease. Further, chronic alcohol consumption was identified as a significant risk factor for cancer in humans. In one such study, alcohol consumption increased the risk of gastric cancer in subjects with gastric atrophy (OR=2.4, p=0.03) (Yamaji et al., 2009) However, the exact mechanism of ethanol-associated carcinogenesis remains unknown (Jelski and Szmitkowski, 2008).

Alcohol is generally metabolized via several different pathways. The breakdown of alcohol by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) is a hallmark of the most common pathway. First, alcohol is metabolized by ADH to a highly toxic substance called acetaldehyde, which is known
to be carcinogenic. Second, the enzyme ALDH then converts the acetaldehyde into non-toxic acetic acid (National Institute on Alcohol Abuse and Alcoholism, 2007). Ethanol is oxidized not only in the liver, but also in the gastrointestinal tract. Although this gastric ethanol metabolism is considerably less than that of the liver, this pathway has important relevance with respect to the first stage (metabolism of ethanol by gastric ADH) of the metabolism of alcohol, and to ethanol-induced tissue toxicity (Seitz et al., 1994). The present study aimed to analyze the influence of low concentrations of ethanol on cell viability, apoptosis and the expression of ADH of gastric carcinoma cells.

**MATERIALS AND METHODS**

**Cell culture**

Human gastric adenocarcinoma cell lines (MGC803, MGC823, and SGC7901) were purchased from Zhejiang Cancer Hospital. Cells were cultured in RPMI1640 media supplemented with 10% fetal bovine serum (GIBCO, USA), 100 U/ml penicillin, and 100 U/ml streptomycin. Cells were then digested with 0.25% volume trypsin and 0.02% volume of EDTA. Logarithmic growth phase cells were chosen for the next step of the experiment after conventional methods of passage.

**Detection of the effect of ethanol on cell proliferation by MTT assay**

Cells were seeded into 96-well plates at 1×10⁴ cells (100ul)/well and grown in an incubator in 5% CO₂ at 37°C for 24 h. After this, the cells were treated with ethanol at different concentrations (0.03125%, 0.0625%, 0.125%, 0.25%, 0.5%, 1%, 2%, 4%). Untreated cells (without ethanol) were set as the control group. After culturing for 48 h, 20 ul MTT (0.5%) (Sigma, USA) was added, and the cells were incubated for another 4 h at 37°C. 150 ul DMSO was added to each well after removing the supernatant. After shaking, the samples were dissolved and put aside for 30 min. At this point, the blue-violet crystals were completely dissolved. The absorbance (A) of samples was measured at 490 nm using the Bio-Rad Model 680 Absorbance Microplate Reader. The inhibitory rate (IR) was calculated according to the following formula: IR (%) = (1-Experimental group A/Control group A) ×100%

**Detection of apoptosis by flow cytometry**

MGC803, MGC823, and SGC7901 cells were incubated with or without different concentrations of ethanol (0.125%, 0.25%, 0.5%) for 48 h. Control and treated cells were stained using the Annexin V/pro-pidium iodide apoptosis kit, and the cells were examined under a Cytomics FC500 MPL fluorescence microscope (Beckman Coulter Company, USA). The distribution of cells and the percentage of dye-labeled cells was then determined.

**Expression of ADH by Western blotting**

The concentration of protein in each lysate was determined using the BCA Protein-100 kit. Proteins were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and subsequently transferred onto nitrocellulose membranes. The membrane was incubated with a primary monoclonal anti-ADH antibody (1:1000) (Santa Cruz, USA) and a secondary anti-mlgG-HRP (1:10000) (Santa Cruz, USA). The immune complexes were visualized by the ECL chemiluminescence method.

**Table 1. Percentage of total apoptosis of three gastric adenocarcinoma cells treated by different concentrations of ethanol.**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>0.125% ethanol</th>
<th>0.25% ethanol</th>
<th>0.5% ethanol</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGC803</td>
<td>4.0%</td>
<td>11.8%</td>
<td>16.4%</td>
<td>0.3%</td>
</tr>
<tr>
<td>BGC823</td>
<td>5.4%</td>
<td>6.5%</td>
<td>11.8%</td>
<td>0.8%</td>
</tr>
<tr>
<td>SGC7901</td>
<td>4.0%</td>
<td>6.5%</td>
<td>14.7%</td>
<td>1.3%</td>
</tr>
</tbody>
</table>
Each protein band was analyzed on the Bio-Rad Versa Doc Image Analysis System.

RESULTS

Inhibitory rate of ethanol on gastric carcinoma cell proliferation

The anti-proliferative effect of ethanol (0.03125%, 0.0625%, 0.125%, 0.25%, 0.5%, 1%, 2%, 4%) on gastric adenocarcinoma cell lines (MGC803, MGC823, and SGC7901) was significant after treating the cells with different concentrations of ethanol for 4 h (Fig. 1). Cell proliferation was inhibited by ethanol in a significant dose-dependent manner in all of the three gastric adenocarcinoma cell lines. The IC50 for all samples was between 0.63% and 1.25%, while the inhibitory rates of ethanol (concentration=2.5%) were all above 70%.

Cell apoptosis of ethanol on gastric adenocarcinoma cells

Flow cytometry analysis revealed that ethanol could induce cell apoptosis. Apoptosis induced by ethanol in MGC803, BGC-823, and SGC7901 cells increased in a dose-dependent manner with the increase of ethanol concentration (Table 1, Fig. 2).

Effect of ethanol on expression of ADH

The expression of ADH increased with the increase of alcohol concentration on the gastric adenocarcinoma cells (Fig. 3).

DISCUSSION

The gastric mucosa is vulnerable in that it is always exposed to various types of irritants such as alcohol, resulting in gastric mucosal cell death through both apoptosis and necrosis in a dose-dependent manner (Tsutsumi et al., 2002). Ethanol is able to induce apoptosis in human gastric carcinoma cell lines such as MKN-74 and MKN-28 (Kasagi, 1999). Apoptosis is a highly organized form of cell death that takes place in normal physiological processes, such as development, homeostasis, tissue turnover, and immune response. Apoptosis plays an important role in differ-
ent pathological conditions such as cancer.

Three kinds of gastric cancer cell lines, MGC803 and BGC823 (poorly differentiated gastric adenocarcinoma) and SGC7901 (moderately differentiated gastric adenocarcinoma), were included in the study. It was found that apoptosis induced by ethanol treatment increased with the increase in the concentration of ethanol in all three cell lines. Our results were consistent with other studies that revealed the same results on the effect of ethanol on the other gastric cancer cell lines.

Fig. 2. Effect of low concentrations of ethanol on cell apoptosis of on gastric adenocarcinoma cell lines (MGC803, BGC-823, and SGC7901). In all panels, cells in the lower left quadrant are alive (D3), cells in the lower right quadrant are in early apoptosis (D4), in the upper right in late apoptosis (D2), and cells in the upper left quadrant are dead (D3). A. Untreated MGC803 cells. B. MGC803 cells with 0.125% ethanol. C. MGC803 cells with 0.25% ethanol. D. MGC803 cells with 0.5% ethanol. E. Untreated BGC823 cells. F. BGC823 cells with 0.125% ethanol. G. BGC823 cells with 0.25% ethanol. H. BGC823 cells with 0.5% ethanol. I. Untreated SGC7901 cells. J. SGC7901 cells with 0.125% ethanol. K. SGC7901 cells with 0.25% ethanol. L. SGC7901 cells with 0.5% ethanol.
In humans, the enzyme alcohol dehydrogenase (ADH) is contained in the lining of the stomach and in the liver. The stomach is involved in the first-pass metabolism of alcohol in humans. It does this by catalyzing the oxidation of ethanol to acetaldehyde, which allows for the consumption of alcoholic beverages. ADH activity varies between men and women, between old and young, and among people and cultures from different areas of the world (Parlesak et al., 2002). Moreover, differences can be found in the activities of total ADH, showing ADH was significantly higher in cancer cells than in healthy mucosa (Jelski, 2007; Jelski et al., 2010). Until now, the effect of different concentrations of ethanol on the expression of ADH of gastric mucosa has been unknown. In this study, ADH expression in three human gastric adenocarcinoma cell lines (MGC803, BGC823, SGC7901) induced by different concentrations of ethanol, was examined. ADH expression of gastric adenocarcinoma cell lines increased with the increase in the concentration of ethanol. This suggested that alcohol consumption could induce the expression of gastric ADH in all the three cell lines.

Alcohol consumption is extremely common in modern society. From this study, it was found that ethanol could induce apoptosis of gastric adenocarcinoma cell lines and stimulate the expression of ADH. However, epidemiologic evidence for an association between alcohol consumption and the risk of developing gastric cancer remains conflicting (Mahjub and Sadri, 2007; Shimazu et al., 2008). Further research should be performed, focusing on the relationship between alcohol and gastric cancer, and the mechanisms involving ethanol metabolism in gastric cancer.

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REFERENCES


