

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

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Abstract - Chronic alcohol consumption has been 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%). An 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 was also able to induce the apoptosis of gastric adenocarcinoma cells in a dose-dependent manner. Alcohol dehydrogenase activity of gastric adenocarcinoma cells increased with the increase in the concentration of ethanol. Ethanol inhibited cell viability and 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 of consuming 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. Furthermore, chronic alcohol consumption has been identified as a significant risk factor for cancer in humans. In one such study, alco-

hol 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 Szmikowski, 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 com-

mon pathway. Alcohol is first metabolized by ADH to a highly toxic acetaldehyde which is known to be carcinogenic. 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 lower than that of the liver, this pathway is relevant 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.

METHODS AND METARIALS

Cell culture

Human gastric adenocarcinoma cell lines (MGC803, MGC823, and SGC7901) were obtained 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 the MTT assay

Cells were seeded onto 96-well plates at 1×10^4 cells (100 ul/well) and grown in the presence of 5% CO₂, at 37°C for 24 h. After this, cells were treated with ethanol at different concentrations (0.03125%, 0.0625%, 0.125%, 0.25%, 0.5%, 1%, 2%, and 4%). The 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°. After removing the supernatant, 150 ul DMSO was added to each well. 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:

$$\text{IR (\%)} = (1 - \text{Experimental group A} / \text{Control group A}) \times 100\%$$

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 with the Annexin V/propidium iodide apoptosis kit, and the cells were examined under Cytomics FC500 MPL fluorescence microscope (Beckman Coulter Company, USA). The distribution of cells and the percentage of dye-labeled cells were then determined.

Estimation of ADH expression by Western blotting

The concentration of protein in each lysate was determined using the BCA Protein-100 kit. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose membranes. The membranes were incubated with a primary monoclonal anti-ADH antibody (1:1000) (Santa Cruz, USA) and a secondary anti-mIgG-HRP (1:10000) (Santa Cruz, USA). The immune complexes were visualized by the ECL chemiluminescence. Each protein band was analyzed on the Bio-Rad Versa Doc Image Analysis System.

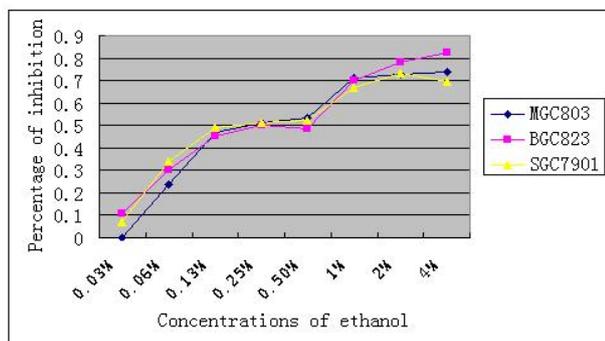
RESULTS

Inhibition of gastric carcinoma cell proliferation by ethanol

The anti-proliferative effect of different concentrations of ethanol (0.03125%, 0.0625%, 0.125%, 0.25%, 0.5%, 1%, 2%, 4%) on the gastric adenocarcinoma cell lines (MGC803, MGC823, and SGC7901) was

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

Cell line	0.125% ethanol	0.25% ethanol	0.5% ethanol	Control
MGC803	4.0%	11.8%	16.4%	0.3%
BGC823	5.4%	6.5%	11.8%	0.8%
SGC7901	4.0%	6.5%	14.7%	1.3%

**Figure 1.** The inhibitory rates of different concentrations of ethanol on gastric adenocarcinoma cell lines (MGC803, BGC-823, and SGC7901). Cell proliferation was inhibited by ethanol in a significant dose-dependent manner in all of the three gastric adenocarcinoma cell lines.

significant after treating the cells for 4 h (Fig. 1). The proliferation all of the three gastric adenocarcinoma cell lines was inhibited by ethanol in a significant, dose-dependent manner. 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%.

The effect of ethanol on gastric adenocarcinoma cell apoptosis

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 in the ethanol concentration (Table 1, Fig. 2).

The effect of ethanol on ADH expression

The expression of ADH increased with the increase of alcohol concentration to which the gastric adenocarcinoma cells were exposed to (Fig. 3).

DISCUSSION

The gastric mucosa is vulnerable in that it is always exposed to different types of irritants, including alcohol, resulting in gastric mucosal cell death through both apoptosis and necrosis in a dose-dependent manner (Tsutsumi et al., 2002). Ethanol is capable of inducing apoptosis in human gastric carcinoma cell lines 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 different pathological conditions such as cancer.

Three 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 in all three cell lines with the increase in ethanol concentration. Our results are consistent with other studies that revealed the effect of ethanol on other gastric cancer cell lines.

In humans, the enzyme alcohol dehydrogenase (ADH) is contained in the lining of the stomach and in the liver. The stomach is involved in first-pass metabolism of alcohol in humans. It does this by catalyzing the oxidation of ethanol to acetaldehyde, allowing for the consumption of alcoholic beverages. ADH activity varies between men and women, between old and young, and among people 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;

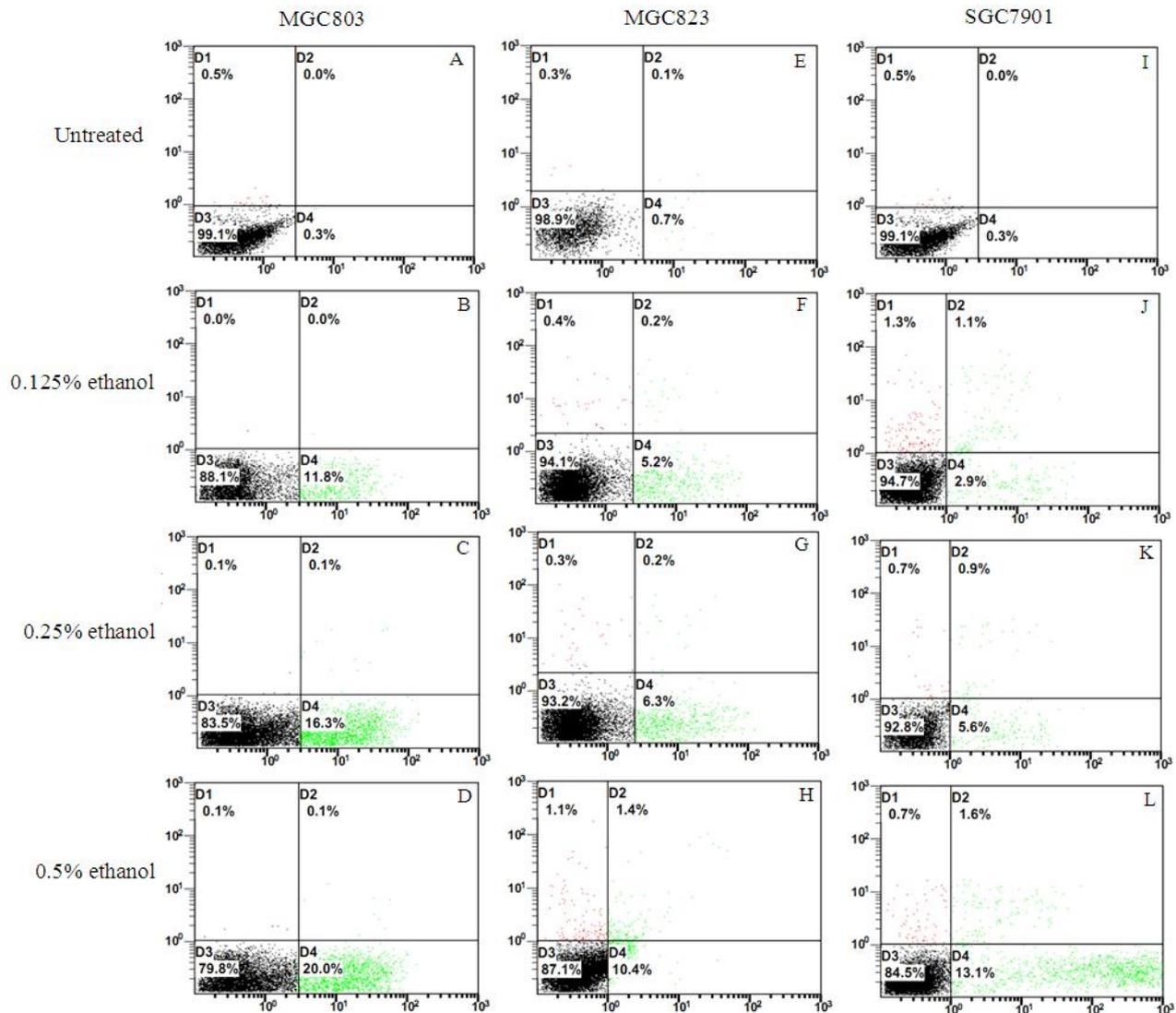


Fig. 2. Effect of low concentrations of ethanol on cell apoptosis of on gastric adenocarcinoma cell lines (MGC803, BGC-823 and SGC7901). In all panels, cells in the lower left quadrant are living cells (D3), cells in the lower right quadrant are in early apoptosis (D4), in the upper right are in late 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.

Jelski et al., 2010). Until now, the effect of different concentrations of ethanol on the expression of ADH of gastric mucosa was 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 ethanol concentration. This suggested that alcohol consumption could induce the expression of gastric ADH in all the three cell lines.

Alcohol consumption is extremely common in many of today's societies. From this study, it was

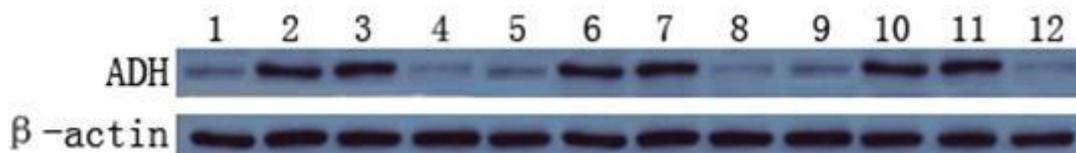


Fig. 3. Effect of low concentrations of ethanol on expression of ADH using Western blot analysis in gastric adenocarcinoma cell lines (MGC803, BGC-823 and SGC7901). The upper figure showed the expression of ADH and the lower figure showed the expression of beta-actin as loading control antibody. Lane1: MGC803 cells with 0.125% ethanol; Lane 2: MGC803 cells with 0.25% ethanol; Lane 3: MGC803 cells with 0.5% ethanol; Lane 4: Untreated MGC803 cells; Lane 5: BGC823 cells with 0.125% ethanol; Lane 6: BGC823 cells with 0.25% ethanol; Lane 7: BGC823 cells with 0.5% ethanol; Lane 8: Untreated BGC823 cells untreated; Lane 9: SGC7901 cells with 0.125% ethanol; Lane 10: SGC7901 cells with 0.25% ethanol; Lane 11: SGC7901 cells with 0.5% ethanol; Lane 12: Untreated SGC7901 cells. From this figure, it can be seen that the expression of ADH increased with the increase of alcohol concentration available to the gastric adenocarcinoma cells.

found that ethanol could induce the 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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