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ORIGINAL ARTICLE

The effect of oxamflatin on the E-cadherin expression in gastric cancer cell line

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Gastric cancer is among the leading causes of cancer-related death, and the symptoms are commonly characterized in advanced stages. Histone acetylation is among the most important epigenetic alterations occurring during cancer development. In addition, reduced E-cadherin expression is a major contributor in the process of tumor cell invasion and metastasis. Oxamflatin is a histone deacetylase inhibitor that has been suggested as a promising anti-tumor agent; yet its effect on the viability and invasion of gastric tumor cells is unclear. We aimed to assess the impact of oxamflatin on the viability of gastric tumor cells and expression of E-cadherin as a marker of tumor invasion susceptibility. In this study, MKN-45 cells were treated with 1, 2.5 and 5 mM oxamflatin and followed by MTT assay after 24–48 h of incubation. To determine E-cadherin expression in treated cells, total RNA was extracted and reverse transcribed to complementary DNA, followed by quantitative real-time PCR. MTT results showed that the viability of MKN-45 cells declines with increasing concentrations of oxamflatin. The results of quantitative real-time PCR showed increased expression of E-cadherin following treatment with oxamflatin at the concentration of 2.5 mM compared with 1 mM. The present results showed that oxamflatin can induce E-cadherin expression and also reduce cell viability in the MKN-45 cell line. On the basis of these findings, oxamflatin can be further considered for the prevention of tumor metastasis.

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INTRODUCTION

Gastric cancer is among the most lethal malignancies.[1] It is also the leading cause of deaths in Japan and the fourth cause of death in the Europe.[2] In Iran, it has been estimated that around 50% of all cancers are gastric.[3] Gastric cancer has a high rate of mortality in the East, South and Central Asia as well as South America.[1] Gastric cancer is usually diagnosed at an advanced stage of the disease when the use of treatments such as chemotherapy, radiation and surgery is inevitable.[4] One of the main factors in the etiopathogenesis of cancer is the change in the pattern of histone acetylation/deacetylation.[5,6] Histone deacetylase inhibitors have emerged as anticancer agents and are classified into different categories based on their chemical structures, including hydroxamic acid derivatives (triacetin A, vorinostat and oxamflatin), carboxylic acid derivatives (valproate and butyrate), aminobenzamides (entinostat and mocetinostat), cyclic peptides (apicidin and romidepsin) and epoxyketones (trapoxin). Histone deacetylase inhibitors can induce apoptosis, growth inhibition and also inhibit the process of angiogenesis in cancer cells.[7,8] Oxamflatin is a histone deacetylase inhibitor derived from aromatic sulfonamides that have hydroxamic acid groups.[9–11] This drug activates the transcription of *Jun D* genes and upregulates extracellular matrix proteins such as fibronectin. It has also been observed that oxamflatin causes cell cycle arrest in G1 phase in the HeLa cells,[10] and possesses potential for toxicity against a variety of tumor cell lines.[12] E-cadherin is a membrane glycoprotein that is expressed on the surface of epithelial cells. In epithelial tissues, E-cadherin causes calcium-dependent adhesion between cells and contributes to the maintenance of normal tissue structure. Cytoplasmic domain of E-cadherin binds to actin present in cytoskeleton through alpha-catenin and beta-catenin, which is necessary for its function.[13] Reduced expression of E-cadherin has been observed in advanced stages of carcinomas, which may be due to the epithelial–mesenchymal transition process.[2] E-cadherin is a key molecule in the adhesion of adjacent epithelial cells, and its inactivation or reduction is an important trigger in tumor formation and metastasis. Several mechanisms are involved in the regulation of gene expression of E-cadherin, including genetic, epigenetic and transcriptional changes. Previous observations, including data obtained from modeling analyses, have supported the notion that E-cadherin can be considered as a suppressor of tumor invasion. This study was aimed to investigate the effect of the histone deacetylase inhibitor oxamflatin on the expression of tumor-suppressor E-cadherin in gastric cancer cell line MKN-45.

MATERIAL AND METHODS

Cell lines

MKN-45 cell line was obtained from the National Cell Bank of Iran (NCBI, Tehran, Iran) and inoculated in DMEM culture medium, containing 10% fetal bovine serum inactivated by heat and 100 μg ml penicillin/streptomycin mixture, and incubated at 37 °C and 5% CO_2.

Drug treatment and MTT

To perform drug treatment and MTT assay, oxamflatin (Sigma, MO, USA) was purchased, and the concentrations of 1, 2.5 and 5 mM were prepared.

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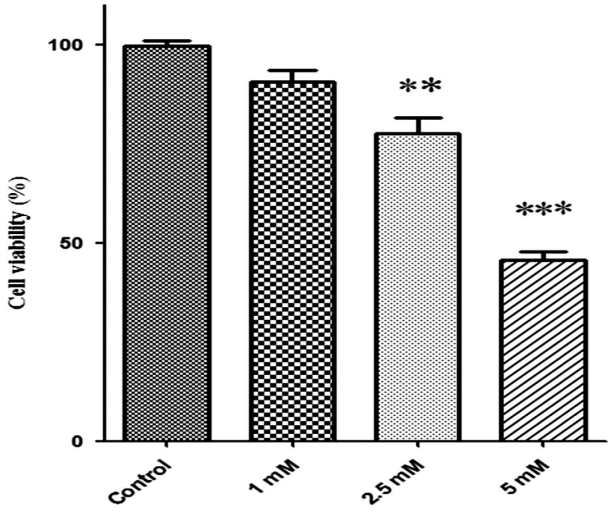

Figure 1. The percentage of MKN-45 living cells exposed to 1, 2.5 and 5 mM oxamflatin after 24 h. $^{**}P < 0.05$, $^{***}P < 0.01$.

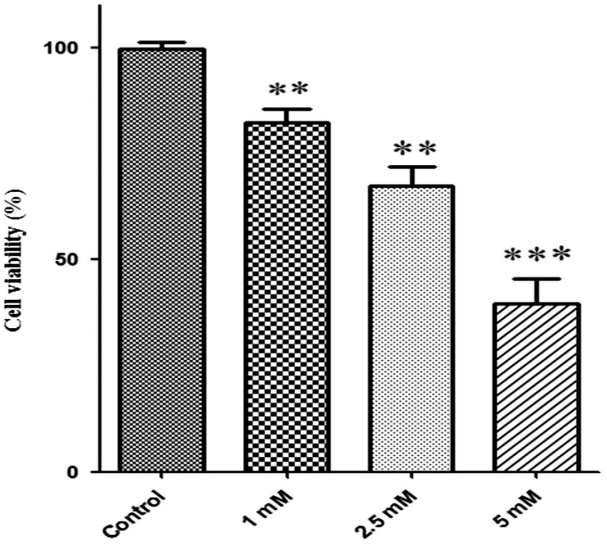

Figure 2. The percentage of MKN-45 living cells exposed to 1, 2.5 and 5 mM oxamflatin after 48 h. $^{**}P < 0.05$, $^{***}P < 0.01$.

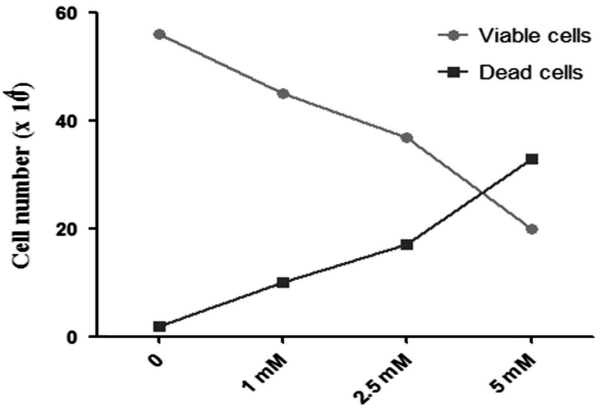

Figure 3. The number of MKN-45 living and dead cells treated with 1, 2.5 and 5 mM oxamflatin after 48 h.

A medium suspension of 100 µl with 5000 cells was added into a 96-well plate. Then, the prepared concentrations of oxamflatin were added to each well, and the plate was incubated at 37 °C in 5% CO_2. Followed by 24 - and 48- h incubation, 100 µl of MTT solution was added to each well, and the plate was returned to the cell culture incubator for 3 h at 37 °C; then the wells were emptied, and 100 µl of DMSO was added and cell density was measured at 570 nm.

RNA preparation

MKN-45 cells were cultured and collected after reaching a high density of 80%, and resuspended at 250 µl of sterile phosphate-buffered saline. Total RNA was extracted from cultured cell using TRIzol reagent (Invitrogen). The purity and integrity of RNA (260/280 nm ratio) was analyzed by spectrophotometer.

cDNA synthesis

RNA extracted with 280/260 ratio between 1.8 and 2 was used to synthesize complementary DNA (cDNA). For cDNA synthesis, 1 µl of RNA, 2 µl of dNTP, 1 µl of random primers six, 1 µl of reverse transcriptase enzyme and 0.5 µl of RNase were poured in sterile microtubes and reached the final volume of 20 µl with distilled water. The microtubes were placed for 10 min at room temperature and then were incubated for 1 h at 42 °C. The reverse transcriptase enzyme was then heat inactivated by 10 min incubation at 70 °C. cDNA products were diluted 10 times with distilled water and were used as DNA template for real-time PCR reactions associated with the expression of target gene.

Quantitative real-time PCR

The evaluation of E-cadherin expression by quantitative real-time PCR was done in the presence of SYBER green master mix. Primers used for the reaction were designed using Oligo Seven software for *E-cadherin* gene (Forward primer: 5′-AGGGGTTAAGCACAACAGCA-3′, Reverse primer: 5′-CTTAGCCTCTCCATCGGACT-3′). A total of 10 µl of SYBR premix Ex Tag II, 0.4 µl of Roxrefrence dye, 0.8 µl of appropriate primers and 2 µl of the diluted cDNA in final volume of 20 µl were poured in sterile microtubes. The thermocycler condition was as follows: 95 °C for 1 min for initial activation, followed by 40 cycles at 95 °C for 15 s, and annealing temperatures 55 °C for 30 s and 72 °C for 30 s using BioRad thermocycler. Finally, the expression level of E-cadherin and glyceraldehyde 3-phosphate dehydrogenase was normalized by $2^{-\Delta\Delta ct}$ method.

RESULTS

Drug treatment and MTT

Drug treatment and MTT were assayed at concentrations of 1, 2.5 and 5 mM oxamflatin during 24 and 48 h, and the amount of living cells was measured in each of the concentrations. The results of oxamflatin effect on MKN-45 cells during 24 and 48 h are shown Figures 1 and 2, respectively.

The number of MKN-45 living and dead cells treated with 1, 2.5 and 5 mM oxamflatin after 48 h using trypan blue staining was evaluated and the results are shown in Figure 3.

E-cadherin expression in MKN-45 cell line treated with oxamflatin

E-cadherin mRNA expression was analyzed by real-time PCR in control and treated MKN-45 cells (Figure 4). These results show that the E-cadherin mRNA was increased in 1 and 2.5 mM oxamflatin-treated cells after 48 h. E-cadherin mRNA was increased 2.5 mM more than 1 mM oxamflatin.

DISCUSSION

The present results showed that oxamflatin can induce E-cadherin expression and also reduce cell viability in the MKN-45 cell line. On the basis of these findings, oxamflatin can be further considered for the prevention of tumor metastasis. There is considerable evidence suggesting that histone acetylation and deacetylation

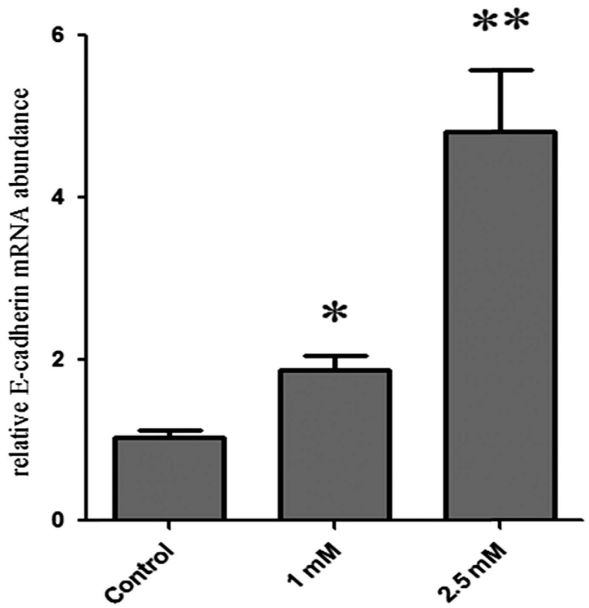

Figure 4. The amount of E-cadherin expression in MKN-45 cells exposed to 1 and 2.5 mM oxamflatin after 48 h. $^{*}P < 0.05$, $^{**}P < 0.01$.

have a significant role in the transcriptional regulation of eukaryotic cells.[5,6] The balance between histone acetylation and deacetylation is an important factor in the regulation of gene expression and cell fate.[14] Histone deacetylase inhibitors have emerged as promising anticancer agents.[5] In this study, we evaluated the effects of one such agent, oxamflatin, on the viability and E-cadherin expression in gastric cancer cells. The findings of MTT assay and total viable cell count using trypan blue method showed a concentration-dependent inhibitory effect of oxamflatin (at the concentrations of 1, 2.5 and 5 mM) on the proliferation of MKN-45 cells. In a previous study, the effect of scriptaid (as a histone deacetylase inhibitor) on the proliferation and survival of normal endometrial epithelial cells and SK-OV-3 ovarian carcinoma cell lines was examined. MTT assay results showed that endometrial cancer cell lines, Ishikawa and SK-OV-3 ovarian carcinoma cell lines, have significant sensitivity to scriptaid, albeit normal endometrial cells showed a low sensitivity to the drug.[15] In the study conducted by Tate *et al.*,[16] the effect of histone deacetylase inhibitor panobinostat was assessed in breast cancerous cell lines. MTT assay was performed to determine the effect of panobinostat on the inhibition of cell growth *in vitro*. The results of MTT assay showed that panobinostat inhibits breast cancer cell growth in a manner. In a research performed by Komatsu *et al.*,[17] the effects of SAHA histone deacetylase inhibitors were studied in lung cancer cells. The cells were incubated with different concentrations of SAHA. MTT assay results showed that the drug can effectively inhibit cell growth in four out of five cells in a dose-dependent manner. The current study examined that *E-cadherin* gene expression changes in the MKN-45 cells following incubation with oxamflatin. The results showed that the expression of E-cadherin is significantly increased when compared with following treatment with oxamflatin. The results of this study are consistent with previous studies suggesting that histone deacetylase inhibitors can be used to regulate gene expression. For instance, in a study conducted by Tsai *et al.*,[18] the effect of valproic acid on *CXCR4* gene expression in mesenchymal stem cells derived from human adipose tissue was investigated. The results showed that an increase in the CXCR4 receptor on the surface of mesenchymal stem cells increases the migration of these cells to damaged tissue. Valproic acid may induce HIF1a in normal oxygenated conditions. The referred study showed that S-valproate and S-diclofenac decrease non-small cell lung cancer proliferation through a mechanism involving histone deacetylase inhibition. The histone deacetylase inhibitory effect of S-valproate and S-diclofenac leads to increased E-cadherin expression while reduced vimentin and ZEB1 expression (a transcriptional suppressor of E-cadherin) and reduced COX-2 activity in non-small cell lung cancer cells.[19] Zhang *et al.*[20] suggested that valproic acid induces the hyperacetylation of histones H3 and H4 and upregulates E-cadherin in prostate cancer cells, by which the E-cadherin–catenin complex dissipates, and cell migration mediated by E-cadherin is inhibited. It has been demonstrated that re-expression of E-cadherin by valproic acid in HPV-positive cell lines is more than that in HPV-negative cell lines. HPV E7 can reduce the expression of E-cadherin, and the restorative effect of valproic acid is related to the augmentation of E-cadherin in HPV-positive cell lines. So, this study demonstrates that valproic acid has more anticancer properties in HPV-positive cell lines, and could potentially be a promising candidate for cervical cancer treatment. *Feng et al.*[21] investigated the effects of valproic acid on the proliferation of cervical cancer Hela cells and tumor growth *in vivo*. They illustrated that valproic acid may act as a HDAC inhibitor or a Notch-signaling activator to suppress tumor development. It was also found that valproic acid upregulates epithelial–mesenchymal transition-associated genes such as *E-cadherin*.[21]

According to the obtained results, it can be concluded that oxamflatin may be used to prevent gastric cancer development and metastasis, and the effective dose of the drug can be examined by performing clinical procedures and *in vivo* and *in vitro* experiments.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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