Effects of Epinephrine on Gastric Adenocarcinoma and Brain Glioblastoma Cancer Cells

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Abstract

Introduction: Gastric cancer is the third cause of death in all malignancies and the fifth most common neoplasm resulting from a combination of specific genetic alteration and environmental factors. Chronic stress can also promote brain tumor cell proliferation and leads to brain metastasis highly resistant to chemotherapy. Catecholamines, norepinephrine, and epinephrine impact neurochemistry and endocrine and immune system functions. The present study investigated the effect of different epinephrine concentrations and β-adrenergic receptor antagonists (propranolol) on proliferation, viability, and adhesion of gastric adenocarcinoma and brain glioblastoma cells.

Material and methods: The human gastric cancer AGS cells and glioblastoma, U87 cell lines were obtained from the Iranian Biological Resource Center (Tehran, Iran) and cultured in RPMI-1640 culture medium supplements. The studied cells were categorized into the nine groups following treatment with epinephrine and propranolol. Wound healing assay (proliferation), Adhesion assay, and cell viability were performed on each group. Graph Pad Prism 6 was used for the statistical analysis.

Results: Proliferation, Viability, cytotoxicity, and adhesion of both cell lines changed under epinephrine agonism in the presence and absence of propranolol (P value<0.001). Epinephrine enhanced the proliferation of both AGS and U87 cells in physiological concentrations, decreased adhesion and viability, and increased cytotoxicity in pharmacological concentrations.

Conclusion: Using a combination of epinephrine and chemotherapy agents in the right stage of developing tumors may have more substantial effects on destroying cancer cells, obtaining the patient's recovery with less repetition of chemotherapy sessions, and curing high-grade cancer tumors.

Introduction

Human and animal studies have shown that chronic behavioral stress may influence tumor initiation and progression through pathways related to the immune system, growth factors, and transcription factors. This is done by activating stress hormones (norepinephrine and epinephrine) and the sympathetic nervous system (1). The correlation between tumor growth, angiogenesis, and metastasis with stress has been demonstrated in numerous clinical and epidemiological studies (2). In acute and chronic stress phases, catecholamines are elevated and bound to adrenergic receptors (3, 4). The α and β adrenergic receptors are widely expressed in mammalian tissues, and the epinephrine and norepinephrine mediate stress responses through the sympatho-adrenomedullary system (5). The effect of catecholamines on target cells through β-adrenergic receptors has been shown in several cancers, including breast and ovary cancer cells (6, 7). Following the stimulation of β-adrenergic receptors, the intracellular cAMP levels change, affecting cell proliferation and differentiation (8). β-adrenergic receptor antagonists block stress-induced enhancement of tumor progression and metastasis in different models of cancers such as breast and prostate carcinomas and malignant melanoma and leukemia in mice (9, 10). Norepinephrine has also been shown to be effective in epithelial-mesenchymal transformation as a crucial process in

tumor metastasis and invasion in different malignancies, including prostate, ovary, gastric, colorectal, and lung cancer. Also, norepinephrine decreases CXCR4 expression and invasion through β2-adrenergic receptors in breast cancer cells. The complexity of the β2-adrenergic receptors signaling pathway may play a role in this unexpected phenomenon (11).

Gastric cancer is the third cause of death of all malignancies and the fifth most common neoplasm worldwide (12). It has been estimated that adenocarcinomas are the most prevalent type of all malignant gastric tumors (about 95%), which originate from surface mucus-producing cells rather than deep acid-producing cells of the gastric mucosa. The remaining 5% consist of lymphomas, stromal, and other rare tumors (13). The clinical outcomes of gastric cancers remain poor due to the lack of a reliable and non-invasive screening test. Therefore, many cases are diagnosed in the late stages (14). Gastric cancer is the result of a combination of specific genetic alternation and environmental factors (15). Studies have also revealed a link between stress and depression with gastric cancer (16, 17).

Glioblastoma multiforme (GBM)-the fourth stage of astrocytoma- is the most common primary malignant tumor of the central nervous system (CNS) that occurs in 1/10,000 patients (18). Despite advances in treatment, including surgery combined with chemotherapy and adjuvant radiotherapy, the prognosis of GBM remains poor as patients succumb within 14 months after diagnosis (19). Studies have shown that β-adrenergic receptors (β-AR) are closely associated with the occurrence and development of brain tumors. A β-AR agonist with phosphorylation (ERK1 / 2) (signal-related kinase phosphorylation) may increase the expression of MMPs to help promote the glioblastoma cell line proliferation (U251), which this effect is blocked by the β-ARs antagonist (propranolol) (20). The present study examined the effect of epinephrine and its antagonist (propranolol) on proliferation, viability, and adhesion of the AGS cell line of gastric adenocarcinoma and the U87 cell line of glioblastoma through the β-adrenergic receptor.

Material And Methods

The human gastric cancer AGS cells and Brain glioblastoma U87 cells lines were obtained from the Iranian Biological Resource Center (Tehran, Iran) and cultured in RPMI-1640 culture medium supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin (all Gibco, USA) at 37˚C in a 5% CO2 humidified incubator, at ~ 80 to 90 % confluence, adherent cultured cells were harvested with trypsin at 37˚C for two minutes and used for the subsequent treatment. Epinephrine was obtained from the epinephrine ampoule Product NO: E 1016, Lot No: 124 K8803, CAS No: 51-42-3, and was maintained at 2–8˚ C. All of the studied cell lines (AGS and U87) were categorized into the nine groups which underwent treatment with epinephrine and propranolol: Control group: Non-treated, The treatment groups were as follows: 16µmol/L epinephrine; 64µmol/L epinephrine; 128µmol/L epinephrine; 256µmol/L epinephrine; 16µmol/L epinephrine + 75µmol/L propranolol; 64µmol/L epinephrine + 75µmol/L propranolol; 128µmol/L epinephrine + 75µmol/L propranolol; 256µmol/L epinephrine + 75µmol/L propranolol. All doses were selected based on former studies (21).

Wound healing assay (proliferation)

To estimate the proliferation, cells were cultured in 24-well plates and allowed to adhere uniformly. A wound was scratched through the cells by moving the pipet tip from top to bottom using a sterile pipet tip. The resulting debris was gently removed by washing with PBS, and then cells were treated as explained above. After 12hrs and 24hrs, pictures were taken (Motic optical microscope, model AE 31, Spain), and the cell proliferation was assessed based on the following table (Table 1) (22).

Table 1
Index of wound healing time

cell growth & repair rate	Index of wound healing time
Non-repair	1
Somewhat	2
Half	3
More than half	4
Nearly complete	5
Complete repair	6

Adhesion assay

The percentage of adherent cells was assessed using 0.5mM EDTA + 0.25% trypsin and 2.5mM EDTA + 1.25% trypsin after five minutes after the treatment in each group. The results were compared to the control group (23).

Cell viability

After 12hrs and 24hrs of treatment using the trypan blue exclusion test and hemocytometer slides, the percentage of living cells in all groups was counted and compared to the control group (24).

Statistical analysis

The data were analyzed using PRISM statistical software version 6 (Social Science Statistical Package, version 6.0, Chicago, Illinois, USA). The results were presented as mean (± SD). All statistical comparisons were performed using a two-way analysis of variance, non-parametric T-test, and one-way post-test (ANOVA) followed by Tukey's post comparative tests. (Statistical significance level P-Value < 0.05 was considered significant.

Results

Epinephrine changes the proliferation of cancer cells

The proliferation rate of cancer cells significantly reduced in 128µmol/L and 256µmol/L epinephrine concentrations compared to 16µmol/L and the control group ($P < 0.001$ for U87 and $P < 0.05$ for AGS).

Adding 75μmol/L propranolol to all batches of cells retrieved cell proliferation rate (Fig. 1a). After 24 hours, the proliferation rate of cancer cells in 16μmol/L epinephrine concentration (near to physiological concentration) was significantly enhanced ($P < 0.001$ for U87 and $P < 0.05$ for AGS) compared to 64μmol/L (near to the pharmacological concentration). However, Cell proliferation rates significantly reduced in epinephrine at concentrations of 128μmol/L and 256μmol/L (pharmacological concentrations) ($P < 0.0001$).

Epinephrine changes the adhesion of U87 and AGS cells

After 5 minutes, increasing the concentration of epinephrine decreased the adhesion of U87 and AGS cells compared with the control group. The adhesion of U87 cells and the cumulative concentration of epinephrine significantly decreased at 64μmol/L ($P < 0.01$). Also, 128μmol/L and 256μmol/L concentrations of epinephrine decreased cell adhesion compared with the control group ($P < 0.001$, $P < 0.0001$). For AGS cells, adhesion was reduced by increasing epinephrine concentrations at 128μmol/L and 256μmol/L compared with 16μmol/L ($P < 0.01$). However, adding 75μmol/L propranolol to wells before the insertion of 128μmol/L and 256μmol/L epinephrine reduced the number of cells detached on the plate compared to epinephrine alone (Fig. 4).

In 16μmol/L epinephrine concentrations, the U87 cell adhesion did not significantly change compared to the control group, but the AGS cell adhesion decreased compared to the control group ($P < 0.05$). However, 64μmol/L epinephrine concentrations decreased the U87 cell adhesion significantly compared to the control group ($P < 0.001$). Cell adhesion has shown the same changes at 128μmol/L and 256μmol/L epinephrine concentrations compared to the control group for U87 ($P < 0.001$, $P < 0.0001$) and AGS ($P < 0.0001$) cells. Adding 75μmol/L propranolol to each batch of both U87 and AGS cells in 16μmol/L epinephrine concentrations caused a significant decline in the detached cells compared to 16μmol/L concentrations of epinephrine alone ($P < 0.05$). However, the maximum reduction of U87 and AGS cell adhesion appeared after adding 75μmol/L propranolol to 256μmol/L epinephrine ($P < 0.0001$), compared to 256μmol/L concentrations of epinephrine alone ($P < 0.001$) (Fig. 5).

Epinephrine has cytotoxicity effects on U87 and AGS cells

After 24 hours, cytotoxicity effects on U87 and AGS cells at high epinephrine concentrations increased compared to the low-concentration epinephrine groups. In 128μmol/L and 256μmol/L epinephrine concentrations, cytotoxicity significantly increased in AGS cells compared to the control group ($P < 0.01$) and in U87 cells compared to 16μmol/L epinephrine concentrations ($P < 0.0001$). Cytotoxicity of Epinephrine intensified by the presence of 75μmol/L propranolol in cell culture before adding epinephrine at 16μmol/L concentrations compared to 16μmol/L concentrations of epinephrine alone (for both U87 and AGS cells $P < 0.001$). However, the presence of 75μmol/L propranolol along with high concentrations of epinephrine (256μmol/L) significantly decreased the cytotoxicity of epinephrine (for both U87 and AGS cells $P < 0.05$) (Fig. 6).

Viability of U87 and AGS cells changed by epinephrine and propranolol

After 24 hours, the treatment of U87 and AGS cells with cumulative concentrations of epinephrine devaluated the viability of the cells compared to the control group. The viability of U87 cells in 64μmol/L epinephrine concentrations significantly decreased compared to 16μmol/L epinephrine concentrations ($P < 0.05$). Also, cell viability significantly reduced at 128μmol/L and 256μmol/L epinephrine concentrations compared to 16μmol/L epinephrine concentrations ($P < 0.0001$). The viability of AGS cells was declined considerably in high concentrations of Epinephrine (128μmol/L and 256μmol/L) compared with the control group ($P < 0.01$). However, 16μmol/L epinephrine showed a significant increase in cell viability than the control group ($P < 0.05$). Nevertheless, cell viability reduced significantly when higher concentrations of epinephrine were used in cell culture (128μmol/L and 256μmol/L ($P < 0.001$). The viability of U87 cells was significantly decreased by adding 75μmol/L propranolol to 16μmol/L concentrations of epinephrine compared with 16μmol/L epinephrine alone ($P < 0.0001$). Conversely, adding 75μmol/L propranolol to the AGS cell culture significantly reversed this phenomenon ($P < 0.001$). However, the presence of propranolol alongside epinephrine at concentrations of 256μmol/L compared to epinephrine alone increased cell viability significantly ($P < 0.05$).

Discussion

The present study investigated the effects of physiological and pharmacological epinephrine concentrations on AGS (Stomach Cancer Cells-SCCs) and U87 (Brain Glioblastoma Cancer Cells- BGCCs) cell lines, which included cell proliferation, adhesion, and viability in two levels of proliferation thresholds. (High-speed proliferation rate for AGS cell line, with a survival rate less than one year, and Low-speed proliferation rate for U87 cell lines, with a survival rate more than ten years). The results have shown that epinephrine enhanced the proliferation of AGS and U87 cells at physiological concentrations. However, pharmacological concentrations potentially decreased cell proliferation. High concentrations of epinephrine have demonstrated toxic effects that inhibit the proliferation of both cell lines in-vitro and reduce the tumor size in-vivo. It appears that by increasing epinephrine concentrations more than 64μmol/L, oxidative stress leads to the creation of hydrogen peroxide and reactive oxygen species (ROS), which can justify epinephrine cytotoxicity.

The results of the present study are consistent with Behonick et al. and Costa et al. studies that reported epinephrine might have toxic effects at doses above physiological levels (25, 26).

This indicates the blocking effect of propranolol through β adrenergic receptors that could provide a reverse reaction to epinephrine in both low and high concentrations of the agonist. Dong et al. reported that, after adding norepinephrine, in a concentration-dependent manner to glioma LN229 and U251 cells, due to the expression of both beta-1 and β2-adrenoceptors, proliferation was significantly enhanced and blocked by propranolol as a nonspecific beta-adrenergic receptor blocker, appeared with a specific time and concentration-dependence (27).

Wong et al. demonstrated the effect of epinephrine on the proliferation of HT-29 adenocarcinoma cells was created through both β1 and β2 adrenergic receptors (28). Yamanaka et al. reported that increased

epinephrine levels (10μg/Ml,100μg/Ml) delayed scratch closure among oral squamous carcinoma cancer cells through the inhibition of intracellular cAMP (29). Also, Sivamani et al. stated that under stress conditions, increased epinephrine levels and expression of beta-adrenergic receptors on keratinocytes result in impaired cellular epithelialization and delayed wound healing. On the other hand, treatment with beta-adrenergic antagonists (timolol) significantly increases the epithelial level of the wound (30). Djelic et al. used six experimental concentrations of adrenaline on human lymphocytes (0.01–200μmol/L) and reported that lower epinephrine concentrations had no genotoxic effect on sister chromatid exchange and micronucleus. However, higher concentrations (5μmol/L,50μmol/L, 150μmol/L, and 200μmol/L) decrease the mitotic index and delay the cell cycle due to the production of ROS (31). Rosenberg et al. showed that catecholamines, including norepinephrine (NE), dopamine, epinephrine, and glia at a concentration of 25μmol/L, were toxic on neurons. Oxidative degradation of catecholamines and hydrogen peroxide production and adrenochrome have suggested that endogenous catecholamines may play a role in normal and abnormal cell death. The toxicity of noreppinephrine is blocked by catalase (32).

The present study results showed that in the presence of propranolol, the adhesion of U87 cells increased, and metastasis decreased. The findings of the present study show that at physiological concentrations, epinephrine increases tumor growth and prevents its metastasis; however, its pharmacological concentrations are likely to decrease U87 cell proliferation and further enhance the metastatic state of tumors by increasing ROS. Numerous studies confirm these results. Palm et al. showed that the migration of breast, prostate, and colon cancer cells was enhanced by stress-related neurotransmitters, norepinephrine (NE = 10μmol/L) in vitro, and this effect was restrained by the inhibitor, β-propranolol (10μmol/L) (33). Pu et al. reported that in a study of the PANC-1 pancreatic cancer cell model, epinephrine promotes migration in a dose-dependent manner and contributes to the stress-induced metastasis in PANC-1 cells. Cell migration was significantly reduced by blocking the β-adrenoceptor β2 (34).

The present study showed that propranolol reduced cell viability at low concentrations of epinephrine and decreased the toxic effect of epinephrine at higher concentrations. The data show that in physiological concentrations of epinephrine, although it increases the viability of U87 cells, in pharmacological concentrations, it decreases the cell viability and has toxicity effects. Other studies confirm our findings. Patri et al. indicated that in both brain tumor cell lines, such as neuroblastoma and glioma C6, norepinephrine increased cell viability by restoring the G2 phase of the cell cycle and decreasing the percentage of cell death (35). Zhou et al. reported the toxic effects of epinephrine induced by extracellular chemicals entering cells and disrupting cellular homeostasis and activating mitochondrial signaling cascades due to stress, results in increased steady-state levels of ROS and activation of Bax, caspases, and cellular damage. This can ultimately lead to cell death and reduced survival rates (36). Uchida et al. showed that catecholamines, such as epinephrine above concentrations of 60μmol/L, decreased the number of living cells in the Human Oral Squamous Cell Carcinoma lines due to ROS production and cell cytotoxicity. However, this phenomenon was not seen in non-catecholamines, such as dexmedetomidine. Catalases also reduced the toxicity of this effect in adrenergic agonists (37). As the data in this study indicate the dual effects of high concentrations of epinephrine due to the potential impacts of its

products, this is consistent with the study of Calvani et al. Since ROS production is a long-standing issue in cancer, Its toxic threshold can be an effective strategy to reduce tumor cell viability.

On the other hand, cancer cells increase signaling activation by maintaining moderate intracellular ROS concentration called "mild oxidative stress," enhancing tumor progression by enhancing cell viability and dangerous tumor phenotype. Many chemotherapy treatments kill the cell by increasing the concentration of ROS in the cell (38). Similar to the present study results, Ciccarese et al. showed that ROS acts as a double-edged sword in cancer cells. Increased mtROS production leads to increased mitogenic signals, oncogenic transformation, genomic instability, and evasion of cell cycle inspections. On the other hand, excessive accumulation of H2O2 leads to irreversible protein modification, oxidative damage to lipids and nucleic acids, blockade of proliferative signaling, and ultimately cell death. Therefore, elevated ROS levels may reflect the Achilles' heel of cancer cells that can be therapeutically abused because it may overcome the toxic threshold by a slight increase in ROS levels, leading to mitochondrial crest regeneration and apoptotic cell death. High levels of ROS in cancer cells balance with increased antioxidant defense (39).

In the present study, epinephrine was represented as a double blade sort that has dual effects. It caused tumor destruction at high concentrations and spread tumor cells to other tissues by declining adhesion in low concentrations, increasing tumor size, and inhibiting cell invasion and metastasis. However, this dual effect of epinephrine and its precise mechanism requires further investigation.

In the early stages of the diagnosis, epinephrine toxicity at pharmacological concentrations reduced the growth and proliferation of glioblastoma-derived brain tumors and reduced the invasive probability. As well as using beta-blockers such as propranolol at lower stages of the tumor.

Conclusion

Epinephrine caused tumor destruction at high concentrations and spread tumor cells to other tissues in low concentrations. Using the combination of epinephrine and chemotherapy agents in the right stage of tumor development may have more potent effects on the destruction of cancer cells.

Declarations

Ethics approval and consent to participate:

This study was approved by the Ethics Committee of Mazandaran University of Medical Sciences ***(IR.MAZUMS.REC.1397.1120)***, and all methods of this study were carried out following relevant guidelines and regulations. This project was supported by the chancellor for Research and Technology of Mazandaran University of Medical Sciences and Immunogenetic research center (IRC). (Project Number: 1120)

Ethics approval and consent to participate

The present study didn't include any human participants or animal models. After obtaining a written consent the ethical Committee of Mazandaran University of Medical Sciences approved and permitted the study.

Consent for publication:

Not applicable.

Availability of data and materials:

The datasets used and/or analyzed during the current study, including all relevant raw data, will be freely available to any researcher wishing to use them for non-commercial purposes without breaching participant confidentiality. Data are available from the corresponding author on reasonable request.

Competing interests:

All authors declare that there is no conflict of interest.

Funding:

Not applicable.

Authors' contributions:

MMS, NR, and AN collected the data, JA and AMS performed the statistical analyses, interpreted data, and drafted and revised the manuscript for important intellectual content.

HZ, SAK, and AMS reviewed the analyses and the final version of the manuscript. SAK, JA, and MMS interpreted the data, revised the manuscript for important intellectual content, and approved the final version.

All authors have read and approved the manuscript.

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Figures

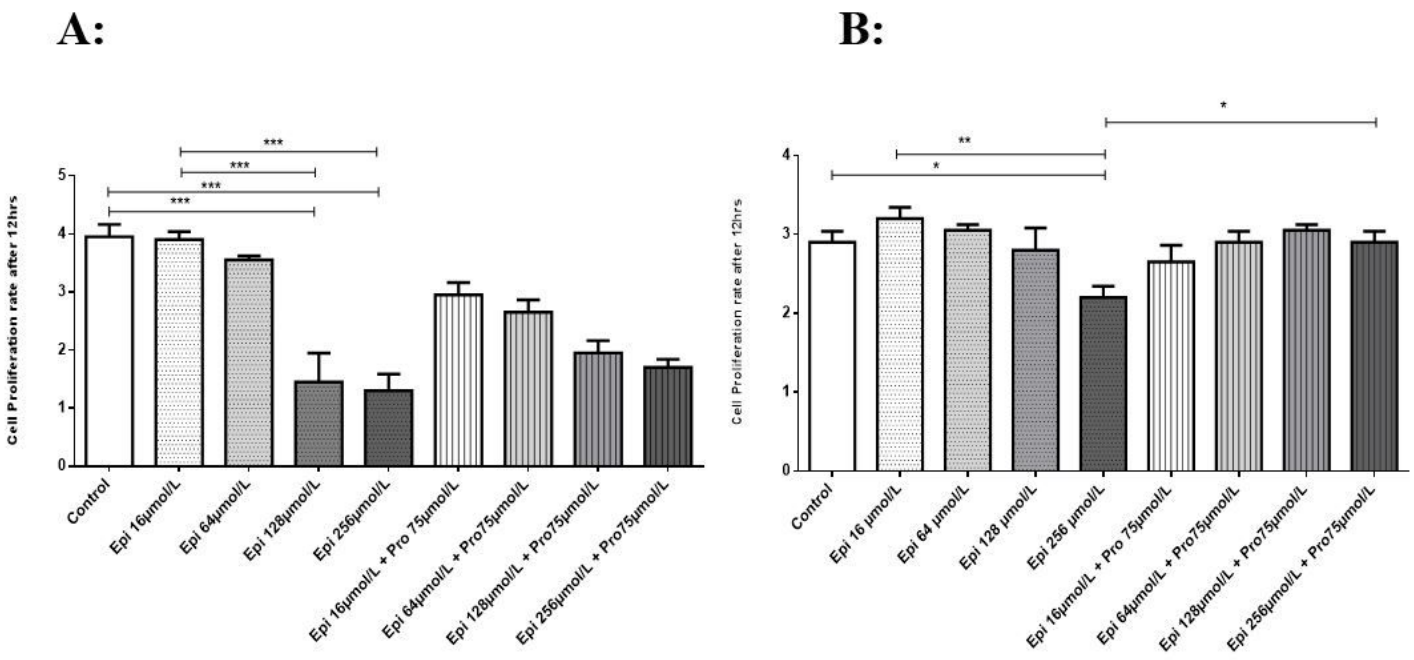

Figure 1

After 12hrs, wound healing assay results. A) U87 cells and B) AGS cells Proliferation treated with epinephrine and propranolol. Data are present as Mean± SD.*P<0.05, **P<0.01, ***P<0.001. Epinephrine (Epi.), Propranolol (Pro.)

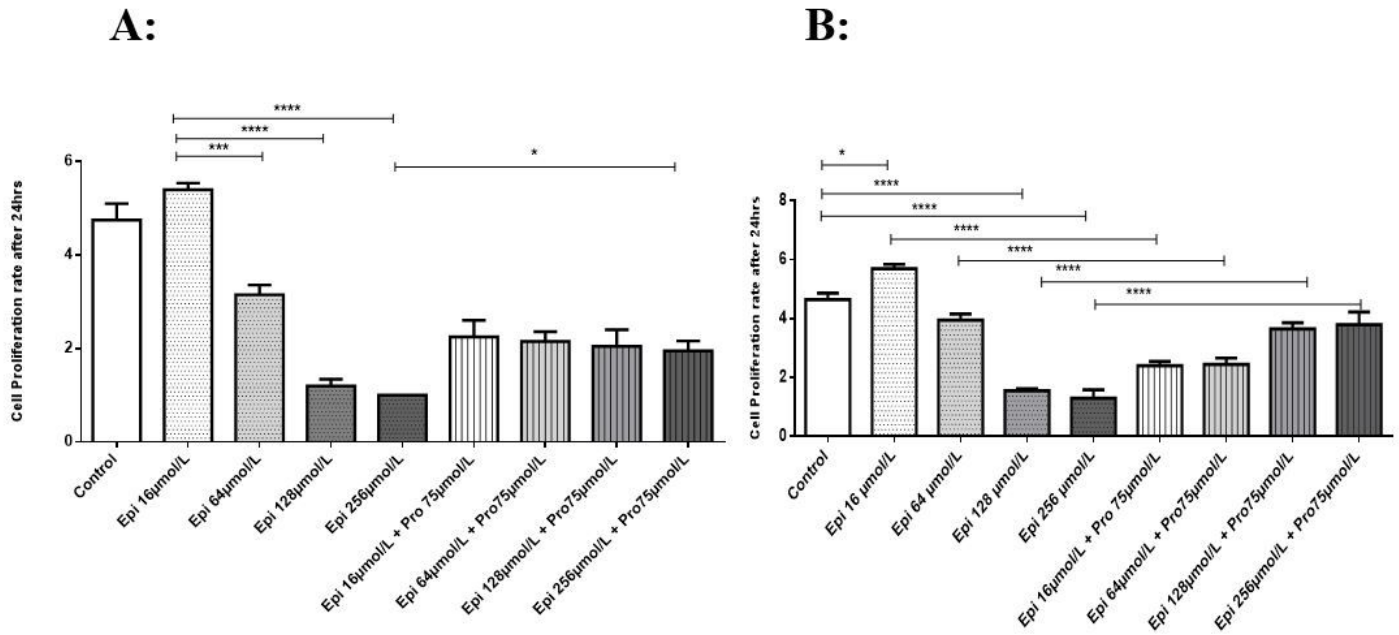

Figure 2

After 24hrs, A) U87 and B) AGS cells proliferation following treatment with epinephrine and propranolol (wound healing assay) Data had shown as Mean± SD.*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. Epinephrine (Epi.), Propranolol (Pro.)

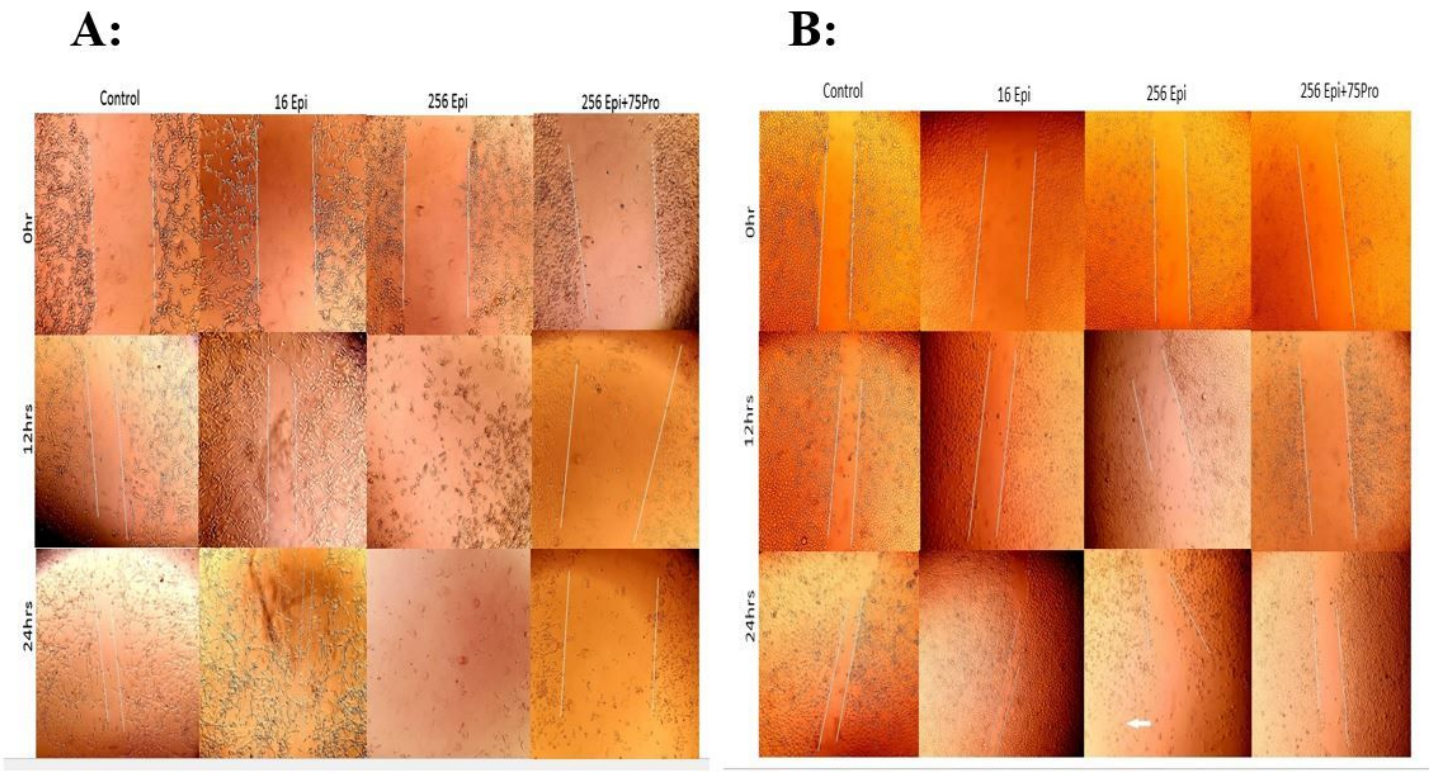

Figure 3

A) U87 and B) AGS cells, The Proliferation after treatment with epinephrine and propranolol (Antagonist). (The lines indicate the rate of the cell growth) for 0hr, 12hrs & 24hrs. Migration and Proliferation of cells examined by scratches in the target groups. Control group was not treated by any medication. Concentration of 16µmol/L showing nearly complete repair of AGS cells after 24hrs (16Epi). Concentration of 256µmol/L was evaluated sharp reduction and incomplete (arrow) for 24hrs (256Epi). Epinephrine group with concentration of 256µmol/L + 75µmol/L propranolol had shown reverse phenomenon (H&E, ×400). Scale bar = 100µm.

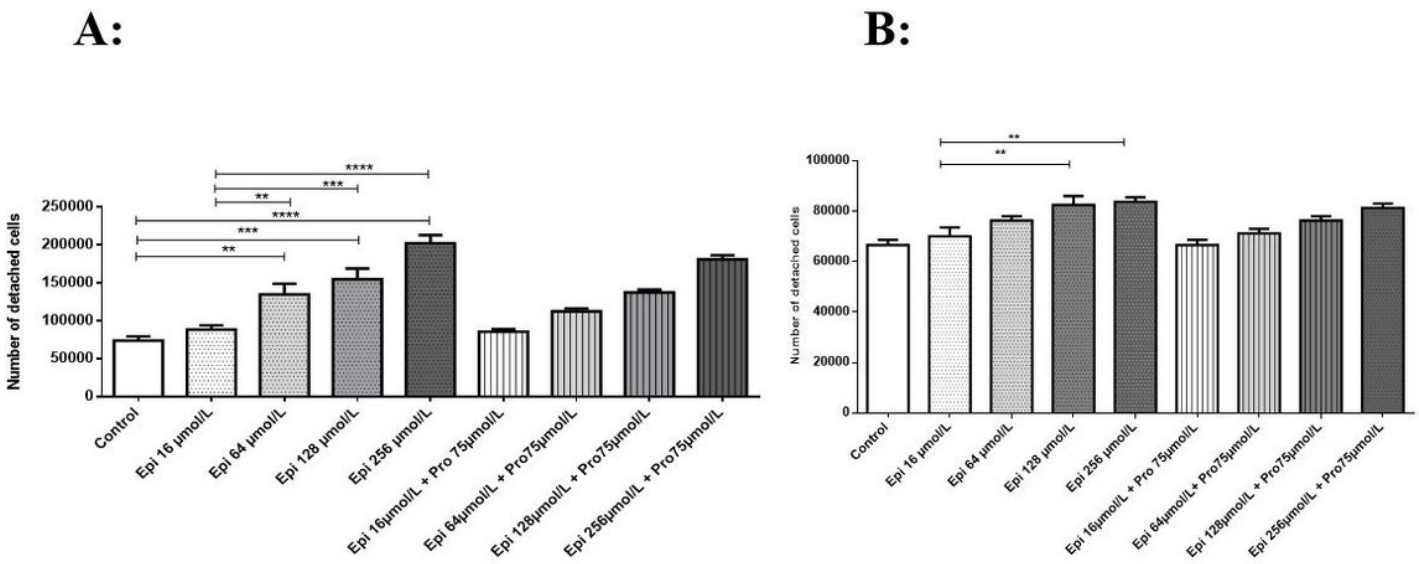

Figure 4

After 5 minutes, A) U87 and B) AGS cells Adhesion treated with epinephrine and propranolol (The number of cells that are detached from the floor of plate). Data are present as Mean± SD.**P<0.01, ***P<0.001, ****P<0.0001. Epinephrine (Epi.), Propranolol (Pro.)

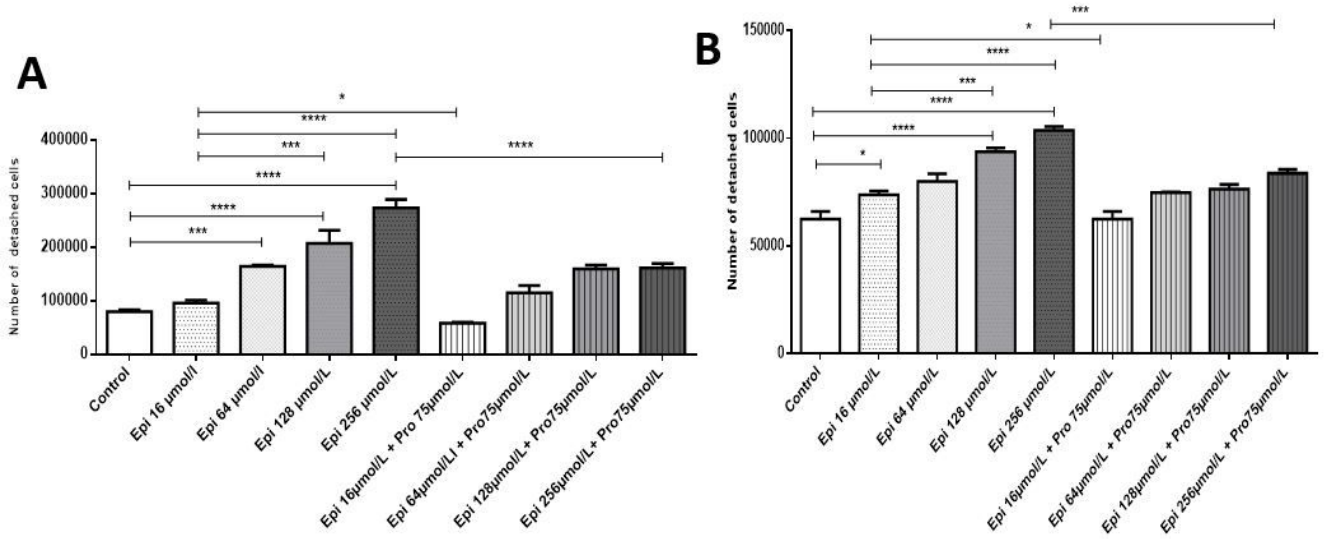

Figure 5

After 30 minutes, A) U87 and B) AGS cells adhesion after treatment with epinephrine and propranolol (The number of cells that are detached from the floor of plate). Data are shown as Mean± SD.*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. Epinephrine (Ep), Propranolol (Pro.)

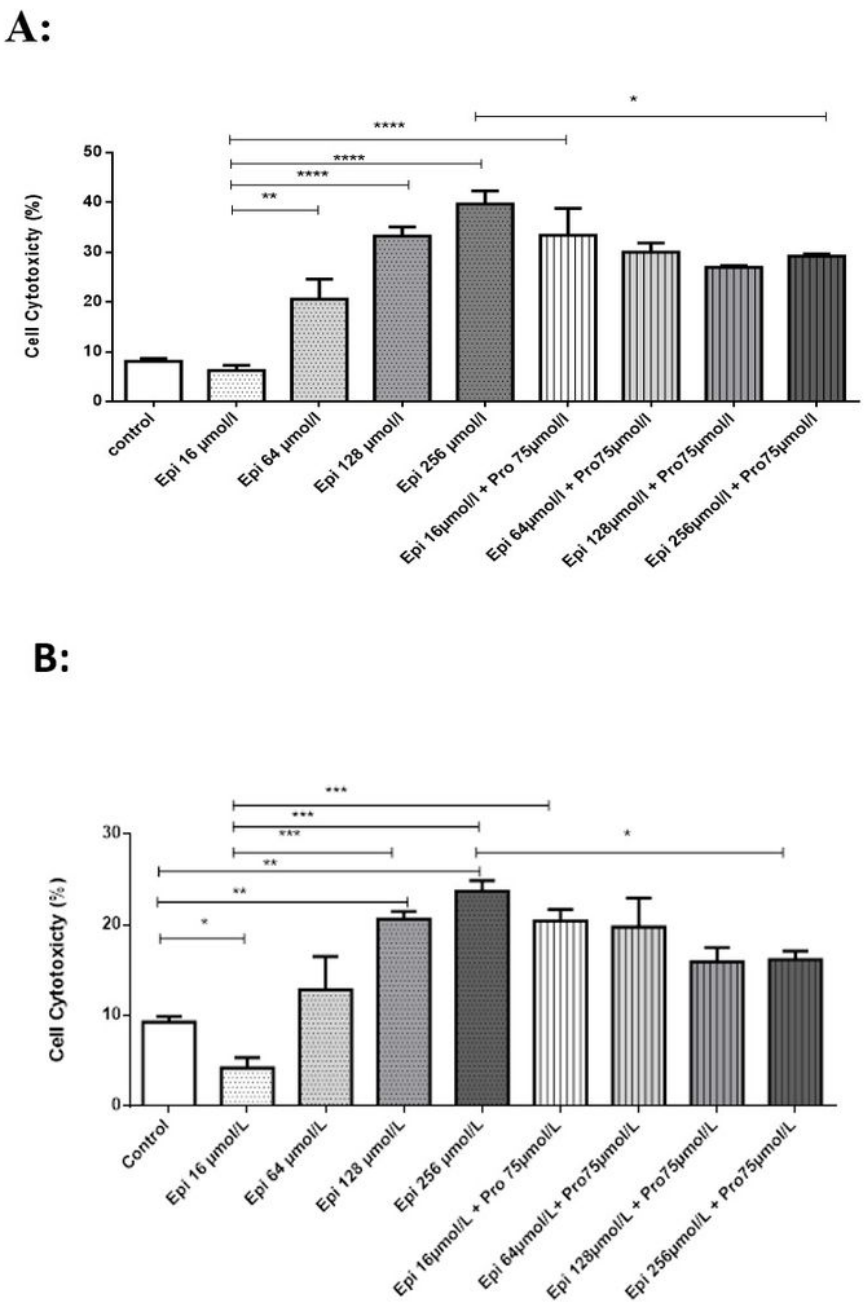

Figure 6

The cytotoxicity of Epinephrine on A) U87 and B) AGS cells following treatment with different concentration of epinephrine and propranolol. Data are present as Mean± SD. * P<0.01, ** P<0.05, *** P<0.001, **** P<0.0001. Epinephrine (Ep.), Propranolol (Pro.)