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ORIGINAL ARTICLE

In vitro effect of carbonic anhydrase inhibitor acetazolamide on cell viability, migration and colony formation of colorectal cancer cells

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Abstract

Acidification of extracellular medium in malignant tumors increases the invasive behaviors of cancer cells. In normal healthy tissues, acid production is catalyzed by carbonic anhydrases. Some of the carbonic anhydrase enzymes are overexpressed in certain types of cancer. The present study aimed to investigate the effect of acetazolamide, a potent carbonic anhydrase inhibitor, on in vitro cultivated cancer cells. Three different assays (MTT test, wound healing and clonogenic assay) were performed using human colorectal adenocarcinoma cells (SW620) to evaluate the suppressive effect of acetazolamide, on the colorectal cancer cells migration ability, colony formation and cell viability. The dose-dependent (1–1000 μM) reducing effect of acetazolamide on the cell viability was more significant within the first 48 h. This inhibitory effect of acetazolamide was found to be decreased at 72 h, and affects cells migration ability of cells at 24 and 48 h. Acetazolamide was observed to inhibit the cell viability, migration and colony formation ability of cells, depending on dose.

Keywords Colorectal cancer · Acetazolamide · Carbonic anhydrase · SW620 · Aquaporins

Abbreviations

ANOVA	analysis of variance
anti-VEGF	anti-vascular endothelial growth factor
AQP	aquaporin
CA	carbonic anhydrase
CO_2	carbon dioxide
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
FBS	fetal bovine serum
5-FU	5-fluorouracil
HCO_3	bicarbonate
H_2CO_3	carbonic acid
IC_{50}	half maximal inhibitory concentration
MTT	3-[4,5-dimethylthiazol-2-yl] -2,5-diphenyltetrazolium bromide
PBS	phosphate-buffered saline
RPMI	Roswell Park Memorial Institute
SD	standard deviation
SPSS	Statistical Package for the Social Sciences

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Introduction

Cancer is one of the most distinct diseases, and is characterized by uncontrolled, rapid and pathological spread of abnormal cells (Nepali et al. 2014). In 2012, 14.1 million new cancer cases and 8.2 million cancer-related deaths were reported around the world (Torre et al. 2015). Colorectal cancer is the third most prolific cancer type worldwide, and ranks fourth among all causes of death. Metastasis is observed in approximately half of all patients with colorectal cancer (Nielsen et al. 2014), tumor cell motility being an important stage in the progression of metastasis, and a positive correlation has been identified between metastatic cancer and increased cell motility. Tumor cells enter into the blood vessels after migrating from the primary tumor, and form a secondary metastatic area by migrating to the secondary tumor region. Currently, there

are no prognostic indicators that can distinguish between metastatic disease and other pathologies (Xiang et al. 2002). Tumor metastasis is the most important stage in carcinomas, and is the direct cause of clinical death (Supuran and Scozzafava 2000a).

Aquaporins (AQPs) are integral membrane proteins that facilitate water flow that can be found in many tissues, and have at least 13 types in mammals. They are implicated in cell migration and angiogenesis, and are effective in transcellular and transepithelial water movement. Tumor cell migration is a major factor in tumor growth and metastasis (Nico and Ribatti 2010). Aquaporin-1 (AQP1), which was first identified as a water channel protein, can be detected in erythrocyte membranes, in the proximal tubule of the kidney, and in the choroid plexus, eye, lung, vascular endothelium, hepatobiliary epithelium and some tumor cells (Benga 2012). Increased levels of AQP1 and actin in tumor tissue can be considered useful prognostic indicators and therapeutic targets (Xiang et al. 2002; Nico and Ribatti 2010). Interestingly, the tissue distribution and even the subcellular localization of AQP1 have a resemblance to carbonic anhydrase (CA), and the two proteins are thought to share structural and functional similarities (Xiang et al. 2002).

Carbonic anhydrase enzymes are metalloenzymes containing Zn^{2+} ions, having first been discovered in bovine erythrocytes (Supuran and Scozzafava 2000a; McCall et al. 2000). Humans have 14 different CA isozymes, although some (CA IV, IX, XII) are overexpressed in tumor cells in cancer (Scozzafava and Supuran 2000; Supuran and Scozzafava 2002). CA catalyzes the hydration of carbon dioxide (CO_2) molecules and the dehydration of bicarbonate ion (HCO_3^-) (Supuran and Scozzafava 2000a; McCall et al. 2000). The acidification of the extracellular environment in malignant tumors increases the invasiveness of cancer cells, and this acidification is caused by CA enzymes ($H_2O + CO_2 <=> H^+ + HCO_3^-$) in normal tissues (Parkkila et al. 2000).

Inhibitors of AQPs, such as heterocyclic and aromatic sulfonamides, and CAs are used in oncogenic events (Supuran and Scozzafava 2000b). It has been suggested that acetazolamide exerts its effect on tumor metastasis by decreasing AQP1 protein expression (Xiang et al. 2002; Nico and Ribatti 2010). Acetazolamide has been used in the treatment of glaucoma for more than 50 years (Xiang et al. 2002), as well as in epileptic seizures, idiopathic intracranial hypertension, altitude sickness, cystinuria, periodic paralysis and central sleep apnea, drug-induced edema, congestive heart failure and in the correction of metabolic alkalosis (Parkkila et al. 2000; Scozzafava et al. 1999). Acetazolamide is different from other sulfa drugs, since it has no anti-bacterial effect and is generally used as sodium salt due to its low solubility in water (Scozzafava et al. 1999). It is an inhibitor of CAs, and shows its inhibitory effect by binding to the active site outside the cell membrane of the CA IX enzyme, which is overexpressed in some tumors, and makes the environment alkaline (Parkkila et al. 2000).

Solid tumor growth is not only characterized by the uncontrolled proliferation of cancer cells, but also by alterations in the tumor area, which can cause the neoplastic mass development and the metastatic spread of cancer cells to distant regions. The excess amounts of proton and CO_2 produced as a result of oncogenic metabolism is stabilized by carbonic acid (H_2CO_3) through the CA enzyme (Neri and Supuran 2011). Many novel CA inhibitors have been found to be effective in suppressing tumor cell growth in vitro in different cell types, such as in the leukemia, non-small cell lung cancer, melanoma, ovarian, kidney, prostate and breast cancer cell lines (Supuran and Scozzafava 2000b). In the present study, we evaluate the inhibitor effect of increasing doses of acetazolamide on cell viability, colony formation and the migration of colorectal cancer cells.

Methods

Cell culture

Human colorectal adenocarcinoma cell line was obtained from the German Cancer Research Center (Heidelberg, Germany), and the cells were cultured under standard conditions (5% CO_2, 37 °C), and Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum (FBS), with L-glutamine (2 mM) used as a cell medium. The cells were kept at −80 °C and centrifuged at 1500 rpm for 5 min at 37 °C until the time of analysis. The formed cell pellet was taken in a fresh medium and then left in the incubator.

Cell passage

The cell passage was implemented in the cells every 3–5 days, according to their frequency in the cell culture bottles. The medium was removed, and the cells were washed with phosphate-buffered saline (PBS), after which, they were treated with 3–5 mL trypsin (0.25% trypsin/ ethylenediaminetetraacetic acid (EDTA) and incubated for 3 min in the incubator, and 3–5 mL of fresh medium was added to neutralize the trypsin. The culture was then centrifuged for 5 min at 1500 rpm and re-suspended at the desired concentration in an RPMI-1640 medium.

Cell proliferation assay

Cell proliferation is based on the staining of living cells with MTT (3-[4,5-dimethylthiazol-2-yl] -2,5-diphenyltetrazolium bromide). The SW620 cells were pre-cultured in the darkness using 96-well plates (Sarstedt, Germany). In the preliminary tests, the SW620 cells were cultured with final concentrations of 1×10^3, 2×10^3, 3×10^3, 4×10^3 and 5×10^3 cells/well.

According to the results of the pre-culture, the concentration of 4×10^3 cells/well was used. Acetazolamide was added at 1 μM, 10 μM, 100 μM, 1000 μM concentrations after 24 h of incubation, and an MTT (Applichem, Germany) solution (5 mg/mL in PBS) was added (10 μL per well) 24 h after the addition of acetazolamide. The plates were then incubated for 3–4 h. After removing the medium, 100 μL of isopropanol (in 0.04 N hydrochloric acid) was added to each well to dissolve the formazan crystals. Optical densities were measured at a 570 nm wavelength using an enzyme-linked immunosorbent assay (ELISA) plate reader (BioTech, USA). MTT was measured with some minor modifications, as described by Eyol et al. (2012).

In vitro wound healing assay

The SW620 cells (60.000 cells/well) were cultured in 24-well plates and subjected to 24 h of standard incubation. As previously described by Liang et al. (2007) and modified by Kaleağasıoğlu and Berger (2014), the cells were drawn with a straight line using a 200 μL sterile plastic pipette tip. Then, they were carefully washed with a culture medium to remove residuals, and 0.5 μL of acetazolamide was added at concentrations of 1 μM, 10 μM, 100 μM, and 1000 μM. The wound healing effect was evaluated at 0, 24, 48 and 72 h.

Clonogenic assay

This measurement is based on in vitro cell survival and proliferation, demonstrating the ability of a cell to colonize, and was carried out after changing some of the protocols reported by Tekedereli et al. (2013). The culture was formed from 500 cells in 2 mL for each of the six well plates, and acetazolamide was added at 1 μM, 10 μM, 100 μM, 1000 μM concentrations after 24 h of incubation, and this was repeated every five days. Approximately two weeks later, the cells were washed with PBS and stained with crystal violet. Colonies containing more than 50 cells were counted and analyzed.

Statistical analysis

The statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 15.0 software (SPSS Inc., Chicago, IL, USA), and descriptive statistics were expressed in mean value ±standard deviation (SD). ImageJ 1.48 software (NIH, Bethesda, Maryland, USA) was used to evaluate the wound healing process. A Shapiro-Wilk test was used to evaluate normally distributed quantitative variables. For the comparison of the groups, a one-way analysis of variance (ANOVA) was used for the independent groups, and a linear regression method was used for the half maximal inhibitory concentration (IC_{50}) calculation. A p value of <0.05 was considered statistically significant.

Results

Cell proliferation assay

In the MTT test, the results obtained at 24, 48, and 72 h were found to be statistically significant. The inhibitory effect of acetazolamide on the SW620 cell line was significant within the first 48 h, although inhibition decreased at 72 h (Fig. 1). This is thought to be due to the environment of 5% CO_2 provided in the incubator to allow the cells to continue their normal life. Although an alkaline medium was obtained through acetazolamide, CO_2 may have reversed this situation. The results of the antiproliferative effect assay showed a significant difference was observed between the control group and the acetazolamide treatment group in all concentrations, except for in the lowest concentration (1 μM) applied at 24, 48 and 72 h.

The survival curves of SW620 cells after acetazolamide administration are shown in Fig. 2. Acetazolamide showed an antiproliferative effect, depending on concentration and time.

In vitro wound healing (scratch) assay

The cells were cultured in a way that there would be 4×10^3 cells in 0.5 mL for 24 well plates for SW620 cell line. After 24 h, they were treated with the indicated concentrations, and images of the cells were taken at 24, 48 and 72 h under an Olympus DP72 microscope. As seen in Fig. 3, the results support the MTT test results. In the wound healing results, the cleft that was opened in the first 24 and 48 h was observed to heal at the lowest level, and the ability of cells to migrate was restrained.

Clonogenic assay

Since counting of colonies with more than 50 cells is not an appropriate method for SW620 cells, cell density was evaluated through photographs (Fig. 4).

Discussion

Although most tumor cells are known to have high vascular permeability and high interstitial fluid pressure, the water pathways in tumors have not been fully clarified. After the discovery of AQPs, which function as highly selective water channels and are a large membrane protein family, the physiological effect of AQPs and their relationship with various diseases with rapid water transport has become a common subject of research, and as a result, AQPs have been identified as potential targets in treatment (Xiang et al. 2002). Both the synthesis of AQPs by acetazolamide and/or the inhibition of

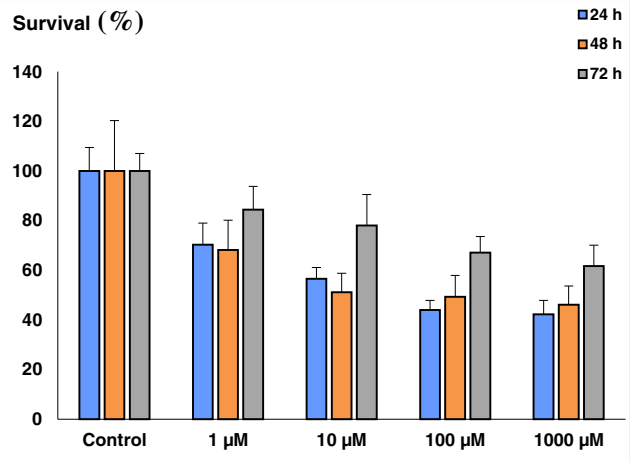

Fig. 1 Survival rates of SW620 cells at different doses (1–1000 µM) at 24, 48 and 72 h after the treatment of acetazolamide

water flow through AQP affect the proliferation of cancer cells, cell migration, metastasis and angiogenic potential (Nico and Ribatti 2010).

Acetazolamide is a drug that is used to reduce intraocular pressure, mainly in glaucoma treatments. Teicher et al. (1993) suggested that acetazolamide treatment may also be beneficial as an adjunct to cancer chemotherapy. It has been further suggested that it shows its effect by inhibiting some CA enzymes (CA IX, CA XII) and AQPs (such as AQP1), which are overexpressed in many types of cancer (Parkkila et al. 2000; Xiang et al. 2004).

Acetazolamide is a classical CA inhibitor. CAs help tumor cells deal with acidic and hypoxic stress through the proton and HCO_3^- convertible hydration of CO_2. In this way, they preserve the physiological intracellular pH despite the acidic environment outside the cell (Neri and Supuran 2011). In addition, acetazolamide has also anti-metastatic effects, having been reported to reduce the number of lung metastases by more than 80% in a mouse model of Lewis lung carcinoma (Xiang et al. 2002) and to reduce metastasis and primary tumor growth in another study with the same mouse model (Li et al. 2007). Acetazolamide has been found to inhibit the rate of invasion in renal carcinoma cell lines (Parkkila et al. 2000), and to have reduced the proliferation of bronchial carcinoid cells (Mokhtari et al. 2013), to inhibit xenograft tumor growth and AQP1expression in colon cancer (Bin and Shi-Peng 2011) and to inhibit the proliferation of T-47D breast cancer cells by inducing autophagia leading to cell death (Mohammadpour et al. 2014). The results of the present study, are consistent with those of previous studies. Depending on the dose, acetazolamide was seen to reduce the cell viability, migration and colony formation ability of SW620 cells.

Although acetazolamide has been found to have the potential inhibit the invasive potential of in vitro cancer cells, its mechanism has not been fully clarified (Parkkila et al. 2000). It has been suggested that acetazolamide, a non-cytotoxic drug can significantly inhibit tumor metastases in vivo significantly (Xiang et al. 2002). An overexpression of CAs has been reported in different human neoplasms, and CAs have been found to be associated with poor prognosis in many types of cancer, such as breast adenocarcinoma and bladder carcinoma (Robertson et al. 2004; Pastorekova et al. 2007). The inhibition of CAs enzymatic activity has been evaluated as a therapeutic strategy against cancer, since CA is an important component in tumor cell survival pathways (Supuran 2008). Acetazolamide or new acetazolamide-derived compounds are used in treatment either separately, or together with aromatic sulfonamides, as CA inhibitors and have been found to inhibit the enzymatic activity of CA IX in human renal carcinoma and cervical cancer cells, to suppress invasive capacity, to reduce cell proliferation and to induce apoptosis by having a high affinity to CA IX (Carlin et al. 2010; Cianchi et al. 2010).

An overexpression of CA IX in many malignant tumors (i.e., lung, cervical carcinoma, esophagus, bladder, breast, and colorectal cancer) has been detected immunohistochemically in Western blot studies. Expressions of CA IX in advanced tumors are found to be higher than at the beginning, and it has been reported to develop resistance to chemotherapy, since it contributes to the extracellular pH becoming more acidic in tumor cells of CA IX (Spugnini et al. 2015). The inhibition of CA IX also increases the efficacy of the anti-vascular

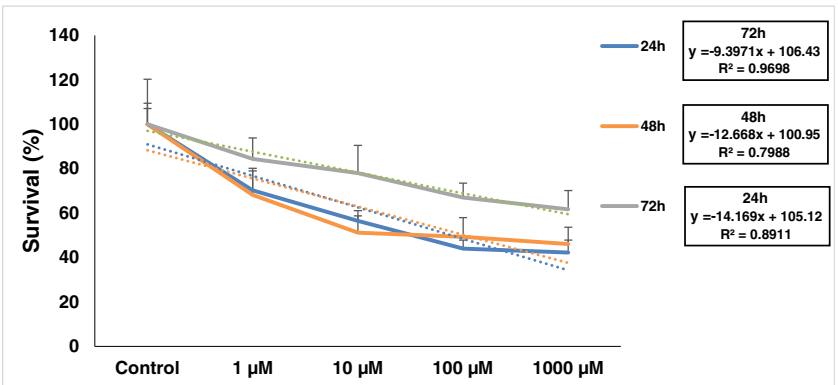

Fig. 2 Survival curves of SW620 cells following exposure to increasing acetazolamide concentrations (1–1000 µM). IC50 values were found to be 528 µM and 795 µM for 24 h and 48 h treatment respectively through linear regression analysis

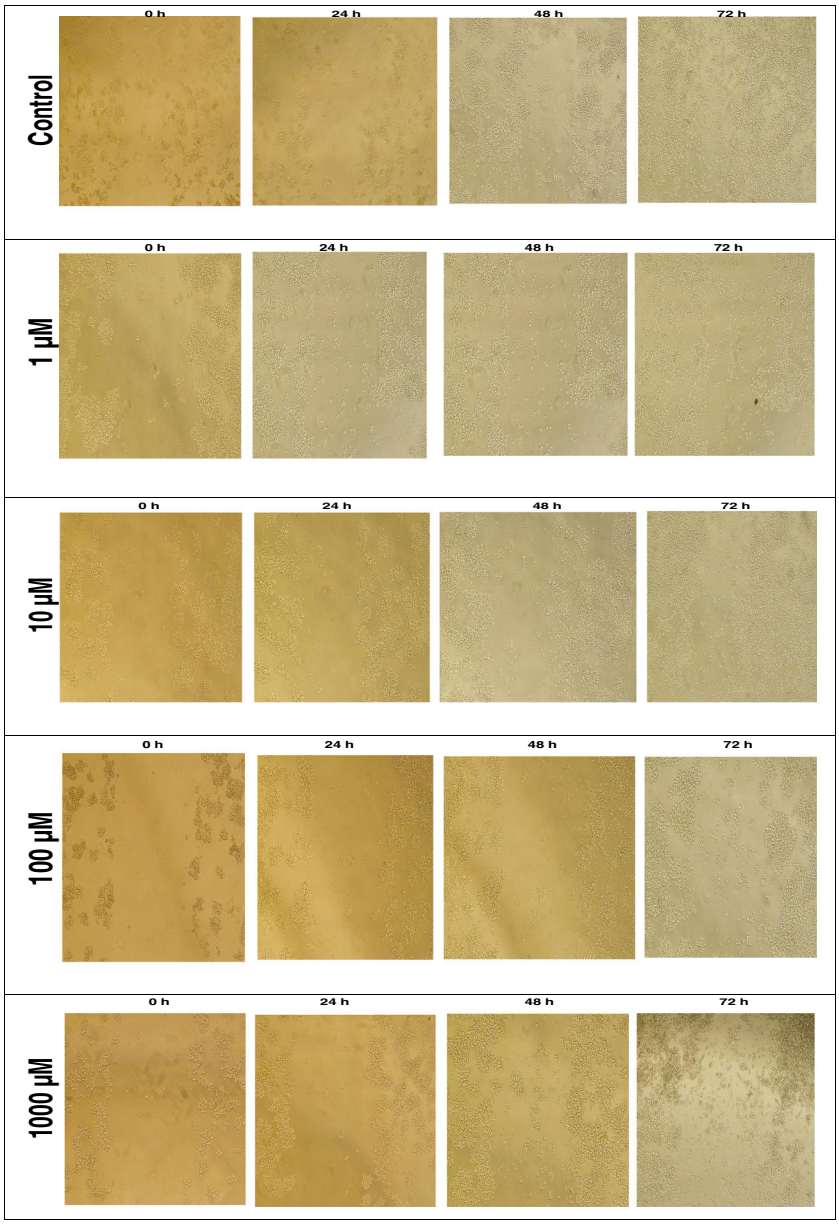

Fig. 3 Wound healing responses of SW60 cell line following exposure to acetazolamide

endothelial growth factor (anti-VEGF) antibody and anti-angiogenic therapy (Pastorek and Pastorekova 2015). CA XII, which is an another tumor associated carbonic anhydrase enzyme, is overexpressed not only in gastric, colorectal and breast cancer, but also in the T-cells of patients with acute lymphoblastic leukemia (De Monte et al. 2014).

As with many types of cancer, colorectal cancer can be treated with surgery, chemotherapy, radiation therapy and palliative care, or a combination of these. Chemotherapeutic agents such as 5-fluorouracil (5-FU), capecitabine, folinic acid (leucovorin), oxaliplatin, irinotecan and tegafur-uracil, or their combinations (FOLFOX, FOLFIRI and CapeOx), are used in chemotherapy (Andre and Schmiegel 2005), although the tumor microenvironment is one of the main implications to be considered when planning treatment. Many tumors develop under hypoxic conditions, and these cause tumor cells to alter the glucose metabolism and produce lactic acid. This leads causes the periphery of tumor cells to be more acidic than normal cells. The cell cycle, proliferation and differentiation process in living cells and tissues are affected by environmental acidity. Similarly, oncogenesis, malignant transformation, metastasis, and angiogenesis are highly affected by environmental conditions. The alkalization of the tumor microenvironment slows down tumor growth and metastasis (Song et al. 2006).

Aquaporin 1 and AQP5 are prominent tumor-related AQPs. In a study, the water permeability of an AQP1 mediated-plasma membrane was reported to be important

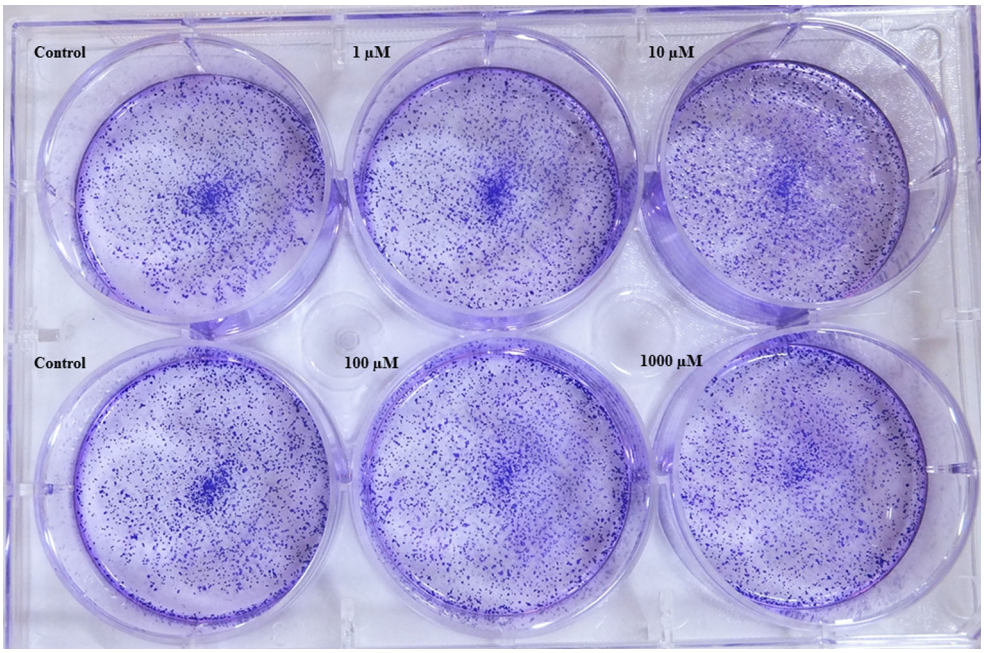

Fig. 4 Acetazolamide treatment reduced clonogenic survival on SW620 cells. Cells were treated for 24 h with increasing doses of acetazolamide, and were then washed with warm PBS, given fresh medium and allowed to grow for two weeks. Cell colonies were measured by staining with crystal violet

for colon cancer cell migration and to be associated with tumor invasion and metastasis. In a tissue microarray analysis of patients with colon cancer, AQP1 was reported to be a prognostic indicator of advanced colon cancer (Yoshida et al. 2013). AQP5, which is another water channel protein, has been reported to promote multidrug resistance in colon cancer cells (HT-29 cells) (Shi et al. 2014). An overexpression of AQP5 has been reported to increase the proliferation, invasion and migration of cells in many human cancer cell lines (Yan et al. 2014).

The MTT reduction activity decreased with acetazolamide concentrations (Fig. 1). The inhibition effect of acetazolamide was significantly detected in the first 48 h, while the inhibition was decreased at 72 h. A statistically significant decrease ($p < 0.05$) in cell viability was observed as the dose increased in the first 24 h (1 µM; 70.2%, 10 µM; 56.5%, 100 µM; 44.0%, 1000 µM; 42.3%). However, it was observed that the cell viability did not significantly change when the dose of 100 µM was exceeded. When cell viability is assessed after 48 h of incubation, 1 µM concentration of acetazolamide inhibited cell viability ratio of 68.2% ($p > 0.05$). Inhibition of cell viability was observed ($p < 0.05$) at increasing concentrations, 10 µM; 51.2%, 100 µM; 49.3%, 1000 µM; 46.1%. Interestingly, the expected effect of long incubation time on cell viability was not observed. After 72 h of incubation, the results were 1 µM; 84.4% ($p > 0.05$), 10 µM; 78.0%, 100 µM; 67.0%, 1000 µM; 61.7% ($p < 0.05$). The dose-response curves presented in Fig. 2 indicate that acetazolamide decreased SW620 cell viability at all concentrations. Exposing cells to acetazolamide 48 h treatment resulted in higher IC50. After 48 h treatment, the IC50 value of acetazolamide increased by 50.6% compared to 24 h treatment. IC50 values were found to be 528 µM and 795 µM for 24 h and 48 h treatment respectively. The micrographs of control, 0–1000 µM acetazolamide at 0, 24, 48, and 72 h were shown in Fig. 3. Cell migration was monitored for up to 72 h. In the wound healing results, the cleft that was opened in the first 24 and 48 h was observed to heal at the lowest level, and the ability of cells to migrate was restrained. The results of wound closure area showed that acetazolamide treatment improved cellular migration which enables significantly accelerated wound closure. As shown in Fig. 4 acetazolamide treatment reduced clonogenic survival on SW620 cells but this reduction is not significant for counting. For this reason the cell density has been photographed.

In conclusion, in this study, acetazolamide was used which is an inhibitor of colorectal cancer associated CAs (CAIX and CAXII) and water channel proteins (AQP1 and AQP5). Our aim was to suppress the migration and invasion of cells by making the microenvironment of the tumor cells alkaline by suppressing the expression of these proteins. In our study, results, acetazolamide was observed to inhibit the cell viability, migration and colony formation ability of cells, depending on dose.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Andre N, Schmiegel W (2005) Chemoradiotherapy for colorectal cancer. Gut 54:1194–1202. https://doi.org/10.1136/gut.2004.062745

Benga G (2012) The first discovered water channel protein, later called aquaporin 1: molecular characteristics, functions and medical implications. Mol Asp Med 33:518–534. https://doi.org/10.1016/j.mam.2012.06.001

Bin K, Shi-Peng Z (2011) Acetazolamide inhibits aquaporin-1 expression and colon cancer xenograft tumor growth. Hepatogastroenterology 58:1502–1516. https://doi.org/10.5754/hge11154

Carlin S, Khan N, Ku T, Longo VA, Larson SM, Smith-Jones PM (2010) Molecular targeting of carbonic anhydrase IX in mice with hypoxic HT29 colorectal tumor xenografts. PLoS One 5:e10857. https://doi.org/10.1371/journal.pone.0010857

Cianchi F, Vinci MC, Supuran CT, Peruzzi B, De Giuli P, Fasolis G, Perigli G, Pastorekova S, Papucci L, Pini A, Masini E, Puccetti L (2010) Selective inhibition of carbonic anhydrase IX decreases cell proliferation and induces ceramide-mediated apoptosis in human cancer cells. J Pharmacol Exp Ther 334:710–719. https://doi.org/10.1124/jpet.110.167270

De Monte C, Carradori S, Secci D, D'Ascenzio M, Vullo D, Ceruso M, Supuran CT (2014) Cyclic tertiary sulfamates: selective inhibition of the tumor-associated carbonic anhydrases IX and XII by N- and O-substituted acesulfame derivatives. Eur J Med Chem 84:240–246. https://doi.org/10.1016/j.ejmech.2014.07.014

Eyol E, Murtaga A, Zhivkova-Galunska M, Georges R, Zepp M, Djandji D, Kleeff J, Berger MR, Adwan H (2012) Few genes are associated with the capability of pancreatic ductal adenocarcinoma cells to grow in the liver of nude rats. Oncol Rep 28:2177–2187. https://doi.org/10.3892/or.2012.2049

Kaleağasıoğlu F, Berger MR (2014) Differential effects of erufosine on proliferation, wound healing and apoptosis in colorectal cancer cell lines. Oncol Rep 31:1407–1416. https://doi.org/10.3892/or.2013.2942

Li XJ, Xiang Y, Ma B, Qi XQ (2007) Effects of acetazolamide combined with or without NaCO3 on suppressing neoplasm growth, metastasis and Aquaporin-1 (AQP1) protein expression. Int J Mol Sci 8:229–240. https://doi.org/10.3390/i8030229

Liang CC, Park AY, Guan JL (2007) In vitro scratch assay: a convenient and inexpensive method or analysis of cell migration in vitro. Nat Protoc 2:329–333. https://doi.org/10.1038/nprot.2007.30

McCall KA, Huang CC, Fierke CA (2000) Function and mechanism of zinc metalloenzymes. J Nutr 130:14375–14465. https://doi.org/10.1093/jn/130.5.1437S

Mohammadpour R, Safarian S, Ejeian F, Sheikholya-Lavasani Z, Abdolmohammadi MH, Sheinabi N (2014) Acetazolamide triggers death induced autophagy in T-47D breast cancer cells. Cell Biol Int 38:228–238. https://doi.org/10.1002/cbin.10197

Mokhtari RB, Kumar S, Islam SS, Yazdanpanah M, Adeli K, Cutz E, Yeger H (2013) Combination of carbonic anhydrase inhibitor, acetazolamide, and sulforaphane, reduces the viability and growth of bronchial carcinoid cell lines. BMC Cancer 13:378. https://doi.org/10.1186/1471-2407-13-378

Nepali K, Sharma S, Sharma M, Bed PM, Dhar KL (2014) Rational approaches, design strategies, structure activity relationship and mechanistic insights for anticancer hybrids. Eur J Med Chem 77:422–487. https://doi.org/10.1016/j.ejmech.2014.03.018

Neri D, Supuran CT (2011) Interfering with pH regulation in tumours as a therapeutic strategy. Nat Rev Drug Discov 10:767–777. https://doi.org/10.1038/nrd3554

Nico B, Ribatti D (2010) Aquaporins in tumor growth and angiogenesis. Cancer Lett 294:135–138. https://doi.org/10.1016/j.canlet.2010.02.005

Nielsen DL, Palshof JA, Larsen FO, Jensen BV, Pfeiffer P (2014) A systematic review of salvage therapy to patients with metastatic colorectal cancer previously treated with fluorouracil, oxaliplatin and irinotecan +/− targeted therapy. Cancer Treat Rev 40:701–715. https://doi.org/10.1016/j.ctrv.2014.02.006

Parkkila S, Rajaniemi H, Parkkila AK, Kivela J, Waheed A, Pastorekova S, Pastorek J, Sly WS (2000) Carbonic anhydrase inhibitor suppresses invasion of renal cancer cells in vitro. Proc Natl Acad Sci U S A 97:2220–2224. https://doi.org/10.1073/pnas.040554897

Pastorek J, Pastorekova S (2015) Hypoxia-induced carbonic anhydrase IX as a target for cancer therapy: from biology to clinical use. Semin Cancer Biol 31:52–64. https://doi.org/10.1016/j.semcancer.2014.08.002

Pastorekova S, Kopacek J, Pastorek J (2007) Carbonic anhydrase inhibitors and the management of cancer. Curr Top Med Chem 7:865–878. https://doi.org/10.2174/156802607780636708

Robertson N, Potter C, Harris AL (2004) Role of carbonic anhydrase IX in human tumor cell growth, survival, and invasion. Cancer Res 64:6160–6165. https://doi.org/10.1158/0008-5472.CAN-03-2224

Scozzafava A, Supuran CT (2000) Carbonic anhydrase and matrix metalloproteinase inhibitors: sulfonylated amino acid hydroxamates with MMP inhibitory properties act as efficient inhibitors of CA isozymes I, II, and IV, and N-hydroxysulfonamides inhibit both these zinc enzymes. J Med Chem 43:3677–3687. https://doi.org/10.1021/jm000027t

Scozzafava A, Menabuoni L, Mincione F, Briganti F, Mincione G, Supuran CT (1999) Carbonic anhydrase inhibitors. synthesis of water-soluble, topically effective, intraocular pressure-lowering aromatic/heterocyclic sulfonamides containing cationic or anionic moieties: is the tail more important than the ring? J Med Chem 42:2641–2650. https://doi.org/10.1021/jm9900523

Shi X, Wu S, Yang Y, Tang L, Wang Y, Dong J, Lü B, Jiang G, Zhao W (2014) AQP5 silencing suppresses p 38 MAPK signaling and improves drug resistance in colon cancer cells. Tumour Biol 35:7035–7045. https://doi.org/10.1007/s13277-014-1956-3

Song CW, Griffin R, Park HJ. (2006). Influence of tumor pH on therapeutic response. B. Teicher (Ed.). Cancer drug discovery and development: Cancer drug resistance. USA: Humana Press Inc. https://doi.org/10.1007/978-1-59745-035-5_2

Spugnini EP, Sonveaux P, Stock C, Perez-Sayans M, De Milito A, Avnet S, Garcìa AG, Harguindey S, Fais S. (2015). Proton channels and exchangers in cancer. Biochim Biophys Acta 1848:2715–2726. doi: https://doi.org/10.1016/j.bbamem.2014.10.015

Supuran CT (2008) Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nat Rev Drug Discov 7:168–181. https://doi.org/10.1038/nrd2467

Supuran CT, Scozzafava A (2000a) Carbonic anhydrase inhibitors and their therapeutic potential. Expert Opin Ther Pat 10:575–600. https://doi.org/10.1517/13543776.10.5.575

Supuran CT, Scozzafava A (2000b) Carbonic anhydrase inhibitors–part 94. 1,3,4-thiadiazole-2-sulfonamidederivatives as antitumor agents? Eur J Med Chem 35:867–874. https://doi.org/10.1016/S0223-5234(00)00169-0

Supuran CT, Scozzafava A (2002) Applications of carbonic anhydrase inhibitors and activators in therapy. Expert Opin Ther Pat 12:217–242. https://doi.org/10.1517/13543776.12.2.217

Teicher BA, Liu SD, Liu JT, Holden SA, Herman TS (1993) A carbonic anhydrase inhibitor as a potential modulator of cancer therapies. Anticancer Res 13:1549–1556

Tekedereli I, Alpay SN, Akar U, Yuca E, Ayugo-Rodriguez C, Han HD, Sood AK, Lopez-Berestein G, Ozpolat B (2013) Therapeutic silencing of Bcl-2 by systemically administered siRNA nanotherapeutics inhibits tumor growth by autophagy and apoptosis and enhances the efficacy of chemotherapy in orthotopic xenograft models of ER (−) and ER (+) breast cancer. Mol Ther–Nucleic Acids 10:e121. https://doi.org/10.1038/mtna.2013.45

Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A (2015) Global cancer statistics. CA Cancer J Clin 65:87–108. https://doi.org/10.3322/caac.21262

Xiang Y, Ma B, Li T, Yu HM, Li XJ (2002) Acetazolamide suppresses tumor metastasis and related protein expression in mice bearing Lewis lung carcinoma. Acta Pharmacol Sin 23:745–751

Xiang Y, Ma B, Li T, Gao JW, Yu HM, Li XJ (2004) Acetazolamide inhibits aquaporin-1protein expression and angiogenesis. Acta Pharmacol Sin 25:812–816

Yan C, Zhu Y, Zhang X, Chen X, Zheng W, Yang J (2014) Down-regulated aquaporin 5 inhibits proliferation and migration of human epithelial ovarian cancer 3AO cells. J Ovar Res 7:78. https://doi.org/10.1186/s13048-014-0078-2

Yoshida T, Hojo S, Sekine S, Sawada S, Okumura T, Nagata T, Shimada Y, Tsukada K (2013) Expression of aquaporin-1 is a poor prognostic factor for stage II and III colon cancer. Mol Clin Oncol 1:953–958. https://doi.org/10.3892/mco.2013.165