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Original Article
Özgün Araştırma

Evaluation of the cytotoxic effect of Sorafenib on HepG2 cell line in different pH environments

Sedat Gökmen[1*], Dilek Güvenç[2]

[1] Kastamonu University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Kastamonu, Türkiye
[2] Ondokuz Mayis University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Samsun, Türkiye

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Abstract: Hepatocellular carcinoma is the third-leading cause of cancer-related deaths. Sorafenib, an FDA-approved multiple kinase inhibitor, is used to treat advanced hepatocellular carcinoma. The microenvironment of cancer cells is acidic (pH 6.6–6.9) due to the Warburg effect. This acidic environment promotes cell survival, proliferation, invasiveness, metastasis, and chemotherapeutic resistance. Our aim was to identify the cytotoxic and antiproliferative effects of sorafenib on human hepatocellular carcinoma at different pH levels. HepG2 cell line was used as a human hepatocellular carcinoma, and different concentrations of sorafenib were applied to HepG2 for 24 hours in media with pH values of 6.6, 6.8, 7.2, 7.6, and 7.8, respectively. The cytotoxic effects of sorafenib were determined with the WST-8 assay. Proliferation was evaluated using live-cell analysis and an imaging system. Sorafenib's inhibitor concentration 50 value was 13.40 µM. Sorafenib showed the strongest cytotoxic effect on HepG2 cells at pH 7.6 ($p<0.05$). According to the proliferation test, sorafenib prepared at pH 7.6 induced a significant decrease (15.84±0.53, $p<0.001$) in proliferation when compared to the control and sorafenib prepared at pH 7.2 and 6.6. This study showed that an alkaline microenvironment increases the cytotoxic and antiproliferative effects of sorafenib.

Keywords: HepG2, proliferation, sorafenib, Warburg effect, WST-8.

Sorafenib'in HepG2 hücreleri üzerindeki sitotoksik etkisinin farklı pH ortamlarında değerlendirilmesi

Özet: Hepatosit karsinomu, kanserle ilişkili ölümlerin üçüncü önde gelen nedenidir. FDA onaylı çoklu kinaz inhibitörü olan Sorafenib, ileri hepatosellüler karsinomu tedavi etmek için kullanılır. Warburg etkisi nedeniyle kanser hücrelerinin mikroçevresi asidiktir (pH 6,6–6,9). Bu asidik ortam, hücrenin hayatta kalmasını, çoğalmasını, invazivliğini, metastazını ve kemoterapötik direncini destekler. Amacımız, sorafenibin insan hepatosellüler karsinomu üzerindeki sitotoksik ve antiproliferatif etkilerini farklı pH seviyelerinde belirlemekti. HepG2 hücre hattı insan hepatosellüler karsinomu olarak kullanıldı ve sorafenib'in farklı konsantrasyonları HepG2'ye sırasıyla pH değerleri 6.6, 6.8, 7.2, 7.6 ve 7.8 olan ortamlarda 24 saat boyunca uygulandı. Sorafenibin sitotoksik etkileri WST-8 testi ile belirlendi. Proliferasyon canlı hücre analizi ve bir görüntüleme sistemi kullanılarak değerlendirildi. Sorafenib'in inhibitör konsantrasyon 50 değeri 13.40 µM olarak bulundu. Sorafenib, HepG2 hücreleri üzerinde en güçlü sitotoksik etkiyi pH 7.6'da gösterdi ($p<0.05$). Proliferasyon testine göre, pH 7.6'da hazırlanan sorafenib, kontrol ve pH 7.2 ve 6.6'da hazırlanan sorafenib ile karşılaştırıldığında proliferasyonda önemli bir azalmaya (15.84±0.53, $p<0.001$) neden oldu. Bu çalışma alkali bir mikroçevrenin sorafenib'in sitotoksik ve antiproliferatif etkilerini artırdığını göstermiştir.

Anahtar kelimeler: HepG2, proliferasyon, sorafenib, Warburg etkisi, WST-8.

Introduction

Hepatocellular carcinoma (HCC) is the most common liver cancer and a major worldwide health issue. HCC ranks fourth globally in terms of cancer-related mortality and sixth in terms of cancer diagnoses (Yu and Ma 2024). Moreover, it is predicted that ~1 million people will be afflicted with liver cancer each year by 2025 (Llovet et al., 2021). The most common risk factors for HCC development are non-alcoholic liver disease, chronic alcohol intake, chronic hepatitis B or C virus infection, and consumption of aflatoxin B1-contaminated foodstuffs (Rich 2024).

Unfortunately, most cases of HCC are diagnosed at advanced stages (or terminal stages), and patients with advanced HCC have limited palliative therapeutic options (Patresan et al., 2024). Sorafenib, an orally multi-targeted tyrosine kinase inhibitor, was the first targeted drug therapy for advanced-HCC clinically approved by the Food and

Yazışma adresi / Correspondence: *Sedat Gökmen, Kuzeykent Mahallesi Orgeneral Atilla Ateş Paşa Caddesi No:19 Merkez, Kastamonu e-mail: sgokmen@kastamonu.edu.tr*

ORCID IDs of the authors: *[1]0000-0002-4793-3030 • [2]0000-0003-0036-0914*

Drug Administration in 2007 (Escudier et al., 2016; Parsons et al., 2017). This molecular-targeted drug is a multikinase inhibitor with antiproliferative and antiangiogenic effects (Yau et al., 2010; Keating 2017). Sorafenib has been shown to have antiproliferative and antiangiogenic effectiveness in different cancer cell lines and experimental animal models (Escudier et al., 2016; Prieto-Domínguez et al., 2016; Keating 2017; Méndez Blanco et al., 2018). It inhibits tyrosine kinase receptors such as vascular endothelial growth factor receptor 2 (VEGFR2), VEGFR3, FMS-like tyrosine kinase 3 (FLT3), platelet-derived growth factor receptor (PDGFR), Ret, and c-KIT, as well as serine/threonine kinases (c-RAF, mutant and wild-type B-RAF) that are involved in tumor cell signaling, angiogenesis, proliferation, and apoptosis "(Yau et al., 2010; Llovet et al., 2021). In normal cells, a significant amount of ATP (approximately 90%) is derived from the oxidation-phosphorylation pathway in the mitochondria (Li et al., 2021). Cancer cells typically display reprogrammed metabolism such they preferentially use the glycolytic pathway to generate energy. Cancer cells metabolize approximately ten-fold more glucose to lactate compared to cells with normal metabolic activity within a certain period (Koppenol et al., 2011). The Warburg effect (or aerobic glycolysis) is characterized by accelerated glycolysis and excessive lactate formation even in the presence of adequate oxygen and was first identified by O.H. Warburg (German biochemist and Nobel Prize winner) in the 1920s (San-Millán and Brooks 2007) . Malignant solid tumours usually have an extracellular pH of 6.5 to 6.9, which is acidic, while normal tissues have an extracellular pH of 7.2 to 7.5, which is much more alkaline (Yuan et al., 2016). This acidic microenvironment is recognized as one of the most important cancer hallmarks (Schwatz et al., 2017). Many recent studies have reported that overall, acidic microenvironments promote tumor cell proliferation, angiogenesis, invasion, metastasis, and chemotherapeutic resistance, as well as escape from immune surveillance and the survival of tumor cells (Wojtkowiak et al., 2011; Esrella et al., 2013; Huber et al., 2017; Tian et al., 2019; Jiang et al., 2020). More importantly, drug efficacy is affected by the pH value of the respective environment. The acidic tumor microenvironment can influence the uptake of drugs into cells. Therefore, the pH of the tumor microenvironment can affect the response of cancer cells to therapy (Justus et al., 2013) .

The regulation of the pH of the tumor microenvironment presents a promising strategy for cancer therapy. Therefore, this study investigated the cytotoxic and inhibitory effects of sorafenib on proliferation at different pH values in human HCC HepG2 cells.

Materials and Methods

Chemicals and reagents

Sorafenib (purity ≥99.9%) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Hydrochloric acid (HCl), sterile dimethyl sulfoxide (DMSO), and trypan blue were obtained from Sigma-Aldrich (St. Louis, MO, USA), and sodium hydroxide (NaOH) was obtained from Merck Chemical Company (Germany). Cell Counting Kit-8 (WST-8, a tetrazolium dye solution) was purchased from Dojindo Molecular Technologies, Inc. (Gaithersburg, MD, USA).

Cell line and culture conditions

The HepG2 cells line (Human hepatocellular carcinoma, ATCC, HB8065) was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cell lines on the cryotube were immediately allowed to dissolve at 37°C in a water bath. The dissolved cells were cultured in Eagle's Minimum Essential Medium (MEM/M-0275, Sigma-Aldrich, St. Louis, MO, USA) supplemented with 5% fetal bovine serum (FBS, 10500064, Gibco, Waltham, MA, USA) in 75 cm^2 plastic flasks. The cells were incubated at incubated at 37°C in a humid atmosphere containing 5% CO_2. The culture medium was changed every 2–3 days until the HepG2 cells reached 70-80% confluence. At this point, the cells were serially subcultured at a ratio of 1:3 by trypsinization (0.25% trypsin/1 mM ethylenediaminetetraacetic acid [EDTA]).

After reaching 80% confluence, the cells were gently detached with 0.25% trypsin− EDTA solution (03-051-5B, Biological Industries) and centrifuged in MEM at 2500 rpm for 5 min, followed by resuspension. Cell suspensions were mixed with 0.4% trypan blue solution in a 1:1 ratio, following which 10 µL of the cell suspension was loaded onto the TC10 System (Bio-Rad Laboratories, Hercules, CA, USA) counting slide and the number of viable cells was quantified on a TC10 automated cell counter (Bio-Rad). The cells (passage 5-8) were transferred successively to a 96-well plate (3×10^4 cells/well) for cytotoxicity assays or a 6-well plate (3×10^5 cells/well) for proliferation assays in the presence of MEM with 5% FBS. The plates were incubated for 24 h in a humidified 5% CO_2 incubator at 37°C. At the next stage, sorafenib was administered to the cells in the

experimental groups. All of the procedures were performed in a sterile laminar flow hood. To observe the effect of different pHs on sorafenib, the pH values of the media were modified to 6.6-7.8 with 0.1 M HCl or 0.1 M NaOH using a pH meter.

Cytotoxicity assay (WST-8)

The WST-8 test was used to evaluate cell viability in accordance with the manufacturer's instructions. First, the half-maximal inhibitory concentration (IC_{50}) at which 50% of the cells were viable was determined. A stock solution of 20 mM sorafenib was created by dissolving it in DMSO. The stock solution was diluted in medium to seven different sorafenib concentrations (5-80 µM) immediately before treatment. In brief, HepG2 cells were transferred into 96-well plates with a total of 3×10^4 cells/well and subsequently treated with 5 to 80 µM concentrations of sorafenib at 37°C for 24 h under a 5% CO_2 atmosphere. After the incubation, 10 µL of WST-8 reagent was added to each well and allowed to incubate for 1 h (37°C, 5% CO_2). The absorbance was read in each well at 450 nm using a microtiter plate ELISA reader (Tecan, software Magellan, Switzerland). Next, the cytotoxic effect of sorafenib at different pH values was assessed by preparing sorafenib at different pH levels and concentration levels. At pH 6.6, 6.8, 7.2, 7.6, and 7.8, HepG2 cells (96-well plates, 3×10^4 cells/well) were treated with different concentrations (IC_{50}, $IC_{50/5}$, $IC_{50/10}$, and $IC_{50/20}$) of sorafenib. These pH values were selected according to the results obtained from an evaluation of the effects of medium pH on the viability of the HepG2 cell line, which was extensively studied in our laboratory (Güvenalp and Guvenc 2020). The treated cells were then incubated at 37°C for 24 h under a 5% CO_2 atmosphere. After 24 h of incubation, the cell viability was measured using WST-8 as previously described. All the treatments were in triplicate, and all the experiments were repeated at least three times. The control in every test was 0.2% DMSO. Absorbance for 100% vitality was measured using untreated cells. The inhibitory concentration of sorafenib was calculated using the absorbance results.

Cell proliferation assay and real-time cell growth analysis

The antiproliferative effect of sorafenib was evaluated over a period of 24 h using the JuLI™ Br Live Cell Analyzer and the JuLI™ Br PC software (both from NanoEnTek, Seoul, Korea). Sorafenib's effect on cell proliferation was assessed at $IC_{50/20}$ concentrations at pH values of 6.6, 7.2, and 7.6, which were significant according to the WST-8 assay results and did not cause cell death. The HepG2 cells were seeded in a 6-well plate at a density of 3×10^5 cells/well and incubated for 48 hours, in order to enable the cells to adhere to the plate. Subsequently, the medium was changed with a new medium with or without sorafenib. The medium without sorafenib was used as the non-treated control. After setting the plate on top of the JULI™ Br Live Cell Analyser, the analyser was put inside the incubator. After choosing a region with a confluence of 40% to 60%, the rate of proliferation was monitored for 24 hours. JULI™ Br images were recorded at 60-s intervals and a real-time cell growth curve was generated using the JuLI™ Br PC software. The proliferation analyses were repeated three times. The increase in percent HepG2 cell proliferation was calculated using the formula below:

$$\%\text{Proliferation} = \frac{\%\text{Final Confluence} - \%\text{Initial Confluence}}{\%\text{Initial Confluence}} \times 100$$

Statistical analysis

Data was presented using descriptive statistics as means and standard error (SEM). The normality of the data was evaluated using the Shapiro–Wilk test. Comparisons of dosages and pH values in each experiment were made using one-way analysis of variance (ANOVA) and Dunnett's or Tukey's post hoc tests for all parameters. The IC_{50} value was calculated in the probit regression analysis. Statistical analyses were performed using IBM SPSS 25.0 (SPSS, Inc., Chicago, IL, USA), and statistical significance occurred when $p<0.05$.

Results

Sorafenib effectively reduced cell viability in HepG2 cells in a dose-dependent manner (Figure 1). The IC_{50} value of sorafenib for HepG2 cells was 13.40 µM. Cell viability was unaffected by DMSO (0.2%) in comparison to the untreated control ($p>0.05$).

Cytotoxic effects of sorafenib on HepG2 at acidic and alkaline pH

Sorafenib had a significantly higher cytotoxic effect on HepG2 cells compared to the DMSO control ($p<0.001$), excluding the $IC_{50/20}$ of sorafenib at pH 6.6 and 7.8 and the $IC_{50/10}$ of sorafenib at pH 6.6. All concentrations (IC_{50}, $IC_{50/5}$, $IC_{50/10}$, and $IC_{50/20}$) of sorafenib at pH 7.6 significantly reduced cell viability by more than 50% (cell viability was about 19-31%). Notably, this decrease was only seen at a pH value of 7.6.

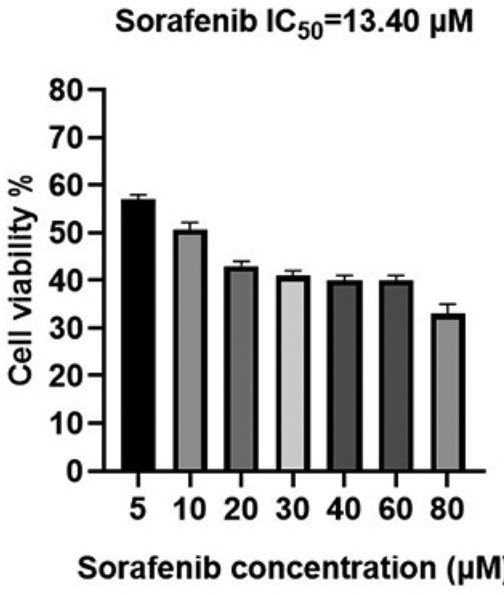

Figure 1. The inhibitory effect of different concentrations of sorafenib on HepG2 cell growth.

Table 1 presents the cell viability of HepG2 cells exposed to sorafenib 24 h after treatment. The WST-8 assay results showed that the cytotoxic activity at different pH levels was generally in the order of pH 7.6>6.8>7.8>7.2>6.6, respevtively. For the IC_{50}, the cytotoxic effect at pH 7.6 did not differ from that at pH 6.8, whereas there was a difference compared to the other groups (pH 6.6 and 7.2: $p<0.001$, pH 7.8: $p<0.05$). For the $IC_{50/5}$ of sorafenib, the cytotoxic effect at pH 7.6 was different from that of the other groups (pH 6.8: $p<0.01$, pH 7.2 and 6.6: $p<0.001$), except for pH 7.8. Additionally, for the $IC_{50/10}$ and $IC_{50/20}$ of sorafenib, the cytotoxic effect at pH 7.6 differed from that of the other groups ($p<0.01$). Sorafenib at pH 7.6 showed significantly higher cytotoxic effects than at other pH values (Figure 2).

Table 1. Viability of HepG2 cancer cells exposed to various concentrations of sorafenib, determined in a WST-8 assay (% cell viability).

pH	MEM	MEM+DMSO	Sorafenib			
			IC_{50}	$IC_{50/5}$	$IC_{50/10}$	$IC_{50/20}$
6.6	100±3.06	95.02±1.03	41.96±2.0[1]a	78.95±2.02[a]	93.64±5.31[a]	98.98±3.03[a]
6.8	100±3.02	93.20±0.44	21.89±0.5[7b]d	51.44±1.49[bc]	53.79±1.33[b]	59.75±1.13[b]
7.2	100±2.58	85.46±1.68	32.63±0.86[c]	67.55±4.97[ac]	72.18±1.02[c]	77.26±1.96[c]
7.6	100±1.54	93.80±2.11	19.76±0.61[b]	20.90±1.39[d]	26.10±1.65[d]	31.35±1.86[d]
7.8	100±4.40	88.86±2.70	25.32±0.48[d]	38.73±3.68[bd]	55.41±5.62[bc]	84.95±1.26[c]

[a,b,c] Different letters in the same column represent statistically significant differences ($p<0.05$). Results are means ± SEM

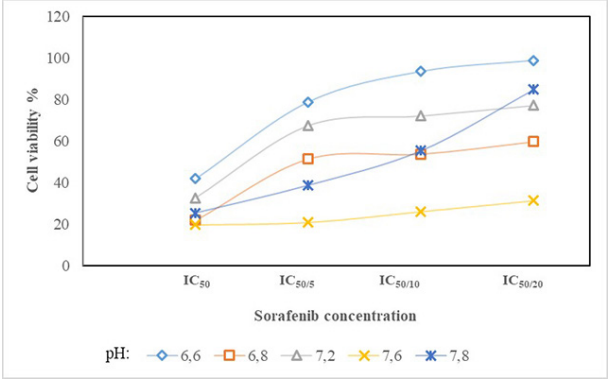

Figure 2. The effect of pH on sorafenib cytotoxicity in the HepG2 cell line was assessed by the WST-8 assay (24-hour exposure).

Proliferation assay

The antiproliferative effect of sorafenib on HepG2 was followed for 24 h at three different pH values: 7.6, 7.2, and 6.6. Cellular proliferation was significantly lower in sorafenib-treated groups than in control groups (Table 2, $p < 0.001$). The proliferation rate in HepG2 cells treated with sorafenib was 27.65%, 19.67%, and 15.84% at pH 6.6, 7.2, and 7.6, respectively. The proliferation assay demonstrated that sorafenib had significantly higher antiproliferative activity against HepG2 cells in the medium with a pH of 7.6 than in the medium with a pH of 6.6 and 7.2 (Table 2, Figure 3). These results suggest that the pH of the microenvironment had an impact on sorafenib's performance.

Table 2. Proliferation rate of HepG2 cells exposed to $IC_{50/20}$ of sorafenib at different pH values.

Group (pH)		Replicate	% Confluence (Initial)	% Confluence (Final)	Proliferation Rate (%)	Average Proliferation Rate (% Mean)
6.6	Control	1	46.07	74.53	61.78	61.13±0.34[a]
		2	48.03	77.14	60.61	
		3	49.17	79.16	60.99	
6.6	$IC_{50/20}$	1	42.81	54.53	27.38	27.65±0.30[b]
		2	43.64	55.97	28.25	
		3	44.97	57.25	27.31	
7.2	Control	1	56.71	81.19	43.17	46.61±0.41[c]
		2	58.87	84.15	42.94	
		3	53.72	77.51	44.29	
7.2	$IC_{50/20}$	1	46.89	56.11	19.66	19.67±0.50[d]
		2	45.47	54.02	18.80	
		3	47.57	57.34	20.54	
7.6	Control	1	48.57	67.42	38.81	38.69±0.77[e]
		2	46.04	64.44	39.97	
		3	49.61	68.11	37.29	
7.6	$IC_{50/20}$	1	52.41	61.25	16.87	15.84±0.53[f]
		2	51.68	59.75	15.62	
		3	50.18	57.73	15.05	

Different letters in the same column indicate a statistically significant difference ($p<0.001$). Results are means ± SEM

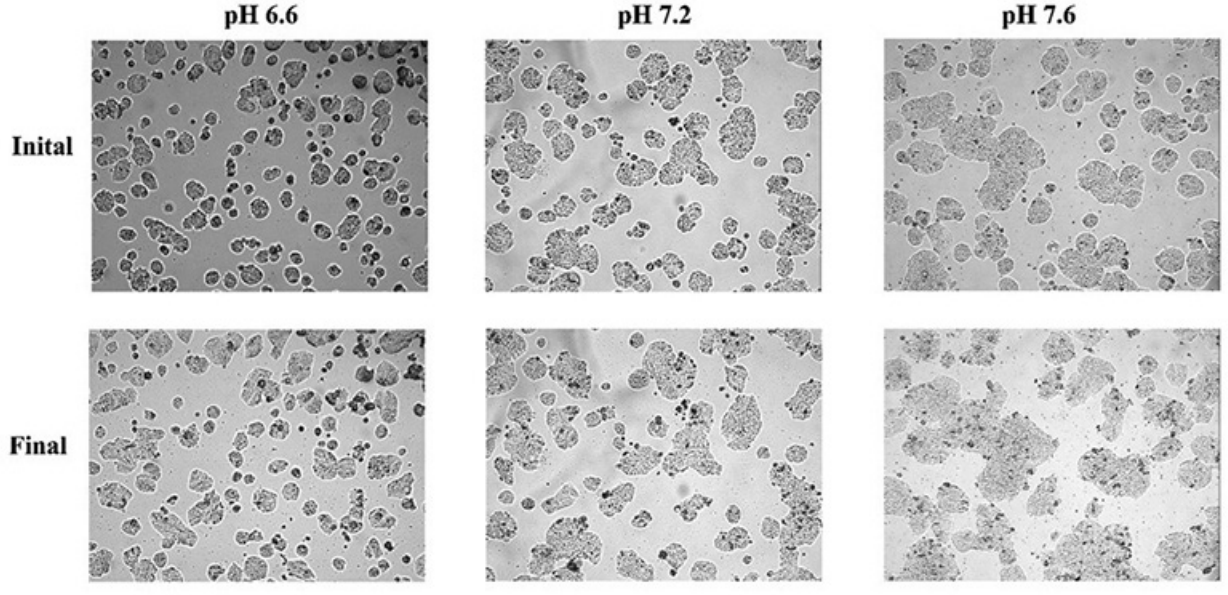

Figure 3. Representative images of HepG2 cells incubated with the $IC_{50/20}$ of sorafenib for 24 hours in culture medium with a pH of 6.6, 7.2, and 7.6. (Initial) 0 hours: cells were imaged by a JuLI™ Br Live Cell Analyzer at 0 hours. (Final) 24 hours: cells were imaged by a JuLI™ Br Live Cell Analyzer at 24 hours.

Discussion

The acidic microenvironment has a significant influence on tumor cell survival and proliferation, and is closely associated with the poor response of tumor cells to chemotherapy (Wojtkowiak et al., 2011; Esrella et al., 2013; Huber et al., 2017; Tian et al., 2019; Jiang et al., 2020). Understanding the effect of the microenvironment pH on chemotherapeutics supports the development of effective therapeutic strategies (Suo et al., 2012; Elsayed et al., 2019). In this study, we evaluated the cytotoxic effect of sorafenib on HepG2 cells at different pH levels. Sorafenib significantly reduced cell viability and proliferation at an alkaline pH of 7.6. Moreover, an acidic pH (pH 6.6) considerably reduced the antiproliferative and cytotoxic effects of sorafenib, except for its IC_{50} group. Thus, we demonstrated here that the activity of sorafenib against liver cancer cells is pH-dependent. In this study, the IC_{50} value of sorafenib was determined using the WST-8 assay (for 24 h) to be 13.40 µM. This value is similar to the range of 10-15.9 µM found in the literature for sorafenib in the HepG2 cell line at 24 h (Suo et al., 2012; Liu et al., 2017; Elsayed et al., 2019). On the other hand, studies reporting IC_{50} values different from the one in the present study were also identified (Raghunand et al., 2003; Liu et al., 2017; Pellegrini et al., 2018). We surmise that this difference may be due to differences in the test method (MTT, MTS, WST-1 or WST-8) and time (24, 48, and 72 h).

It has been recognised that one significant characteristic of solid tumours is an acidic microenvironment (Schwartz et al., 2017). Although it is known that an acidic microenvironment also negatively modulates drug sensitivity, only a few studies have specifically aimed to evaluate the cytotoxic effect of chemotherapeutics on cancer cells under different pH conditions. Therefore, we prepared sorafenib under different pH culture conditions and treated HepG2 cells accordingly. In line with previous reports on other chemotherapeutics, the acidic microenvironment attenuated the cytotoxic activity of sorafenib.

It has been suggested that the cytotoxic effect of chemotherapy may be enhanced by enabling a shift of the microenvironment pH to an alkaline pH in the presence of sodium bicarbonate. As expected, the antiproliferative effects of sorafenib on HepG2 cells increased when the extracellular microenvironment pH value increased from pH 6.6 to 7.6. It is described in the literature that an acidic microenvironment reduces or inhibits the effect of chemotherapeutics e.g., doxorubicin, mitoxantrone, cisplatin, and paclitaxel (Raghunand et al., 2003; Pellegrini et al., 2018). The literature also reports a 17% and 68% reduction in proliferation caused by sorafenib and a 23% and 71% reduction in proliferation caused by sunitinib at doses of 1 and 10 µM, respectively, with a 24 h exposure period in endothelial cells at an alkaline pH (7.4). However, no significant changes were found in the proliferation rate with sorafenib or sunitinib treatment at pH 6.4 (Faes et al., 2016). Similar situation was observed in this study, depending on the pH of the microenvironment to which the cells were exposed. Studies on concurrent cell proliferation showed that sorafenib inhibited HepG2 cell proliferation more at pH 7.6 than at pH 7.2 and 6.6 (pH 7.6: 15.84±0.53%, pH 7.2: 19.67±0.50%, and pH 6.6: 27.65±0.30%; $p<0.001$). These results show that a low microenvironmental pH reduces sorafenib's drug delivery and activity. Therefore, sodium bicarbonate therapy could be an important strategy for overcoming drug resistance.

Compared to their activity at neutral or alkaline pH, many clinically used anticancer drugs exhibit reduced cytotoxicity and antiproliferative activity at acidic pH. The efficacy of various anticancer drugs, including sorafenib, everolimus, doxorubicin and paclitaxel, was investigated in cultured cells grown in a medium with a normal pH (7.3-7.4; HCT116) or in a medium adapted for low pH (6.8) growth (AA-HCT116). Sorafenib and everolimus had similar cytotoxic effects in both HCT116 and low-pH-adapted HCT116 cells at concentrations ranging from 0.352 to 50 µM for 72 h. In contrast, low-pH-adapted HCT116 cells exhibited less sensitivity to other anticancer drugs (Pellegrini et al., 2018). In our study, the effectiveness of sorafenib at pH 6.6 was found to decrease compared to that at pH 7.6. Cell viability was determined to range from 19.76% to 31.35% at the microenvironment pH (7.6) at which sorafenib was the most effective, whereas it ranged from 41.96% to 98.98% in the lowest, acidic pH microenvironment.

Recent studies have shown that sodium bicarbonate can enhance the antitumor activity of chemotherapeutic agents and exhibit anticancer activity. The addition of sodium bicarbonate increases the effect of anticancer drug treatment *in vivo* by shifting the extracellular pH of cancer cells to alkaline. Two hours before mitoxantrone treatment, the administration of a single intraperitoneal dose of sodium bicarbonate (0.7 mL, 1 M $NaHCO_3$) enhanced the antitumor activity of mitoxantrone in C3H/Hen and resulted in about a 45% higher area under

curve compared to the control group ($p<0.01$). In the same study, the researchers also found that $NaHCO_3$ (acute alkalinization: 0.7 mL, 1 M $NaHCO_3$, 2 h before doxorubicin or chronic alkalinization: 200 mM $NaHCO_3$ ad libitum, 48 h before doxorubicin) enhanced doxorubicin administration in both C3H/C3H and MCF-7/SCID systems. However, $NaHCO_3$ (0.5 mL, 1 M intraperitoneal) was found to have no significant influence on the therapeutic efficacy of paclitaxel in tumor-bearing mice (Raghunand et al., 2003). It is reported that $NaHCO_3$ was shown to drastically decrease the growth rate of MC-38 tumour allografts and HT29 tumour xenografts, as well as the number and size of metastases, in a breast tumour xenograft model (Faes et al., 2016). However, contrasting results have also been reported in that sodium bicarbonate had no effect on the proliferation of B16 melanoma tumors in mice (immune-competent, C57BL/6) and MDA-MB231 breast tumors in human tumor xenografts (Thews et al., 2006; Estrella et al., 2013; Pilon Thomas et al., 2016). In this study, the cytotoxic effect of sorafenib at the lowest concentration at pH 7.6 was higher than that of the highest drug concentration at pH 6.6. These results are supported by persuasive studies showing that low-pH-adapted cells are resistant to many chemotherapeutics. Moreover, it has been suggested that lowering the drug dose with an alkaline microenvironment may also be effective in reducing the adverse effects of sorafenib.

Even though the pH of the environment is alkaline, cancer cells may show a decrease in the percentage of cell viability and proliferation rate. Cell viability at pH 6.8 (64.82%) was similar to that of the control (86%). On the other hand, the results indicated that cell viability increased at pH 6.6 while significantly decreasing at pH 7.8 compared to that at pH 7.2 (Guvenalp and Guvenc 2020). Similarly, the cell viability of EC and human gastric cells (SGC-7901 and MKN45) was suppressed at an alkaline pH (7.4-8.0) compared to an acidic pH (6.0, 7.0, 6.6, and 6.8), and proliferation was suppressed by 35-65% (Li et al., 2020). In this study, we have shown that the proliferation rate of HepG2 cells at pH 7.6 (38.12%) was very low when compared to that at pH 7.2 and 6.6 (43.72% and 60.47%, respectively). These results support other studies claiming that alkaline treatments (e.g., sodium bicarbonate) may complement cancer treatment.

It is also known that the tumor microenvironment affects the cell death mechanisms (such as apoptosis and autophagy) in cancer cells. A low extracellular pH can increase cell survival by preventing apoptosis in various cancer cells. It was reported that apoptotic cell death was dramatically reduced in lymphoma (WEHI7.2, CEM-C7, and S49), breast cancer (MDA-MB 231), and gastric cancer (SGC-7901 and MKN45) cells in acidic culture medium (pH 6-6.7). It was also reported that the apoptotic rate of cells gradually increased with an increase in pH; consequently, an alkaline environment (pH 7.4-8) induces apoptosis (Rabiee etal., 2019; Li et al,. 2020).

The number of novel and innovative cancer treatments has been increased recently, providing fresh hope for effective cancer treatment. However, multidrug resistance is the leading cause of chemotherapeutic failure in the treatment of cancer, affected by drug efflux pumps such as phosphorglycoprotein (P-gp). The acidic microenvironment can increase the expression and activity of P-gp in cancer cells by up to ~70%. The activity of P-gp was reported to be significantly elevated (doubled) at pH 6.6 compared to pH 7.6 in rat R3327-AT1 prostate carcinoma cells (Thews et al., 2006). In a similar study, the activity and expression of P-gp did not change significantly under normal pH (7.5) conditions but increased by 70% under acidic pH (6.6) conditions in human colorectal carcinoma cells (LS513) (Lotz et al., 2007). Our data clearly demonstrated that sorafenib showed less antitumoral efficacy at pH 6.6 than at pH 7.6 in HepG2 cells. P-gp may be one of the reasons why HepG2 cells are more resistant to sorafenib at an acidic pH, even if this was not immediately evident.

Conclusions

In conclusion, sorafenib has the most cytotoxic and antiproliferative effects on HepG2 cells at pH 7.6. It is worth noting that an acidic microenvironment (pH 6.6 and 6.8) has been linked to a reduced cytotoxic effect of sorafenib. In light of this observation, some studies have advised using alkaline therapy to reverse the pH gradient and increase chemotherapeutic effects. However, there is no conclusive clinical data linking alkaline therapy to a favorable outcome for anticancer therapy. Further research is needed to determine the link between an alkaline therapy and cancer.

Ethics Committee Approval: This study is not related any experimental animal study, thus, is not required approval of Animal Experiments Local Ethics Committee.

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