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RESEARCH

Could the local anesthetics levobupivacaine and ropivacaine be used to treat colon cancer?

Lokal anesteziklerden levobupivakain ve ropivakain kolon kanseri tedavisinde kullanılabilir mi?

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Abstract

Purpose: Colon cancer is among the most common causes of death among cancers, and in the treatment of colon cancer, adjuvant chemotherapy is applied mainly after curative surgery, to prevent recurrence and metastases after resection. In recent years, local anesthetics have received increasing attention in cancer research. This study aimed to investigate the proliferative and apoptotic effects of local anesthetics levobupivacaine and ropivacaine on SW480 colon cancer cells.

Materials and Methods: In the study, different concentrations and durations of levobupivacaine and ropivacaine were applied to the SW480 colon cancer cell line and their effect on proliferation was determined by MTT analysis. The TUNEL method was used to determine its apoptotic activity. The results were evaluated statistically.

Results: It was determined that levobupivacaine and ropivacaine decreased cell proliferation in SW480 colon cancer cells depending on dose and time. It was also observed to promote apoptosis in colon cancer cells.

Conclusion: According to the study data, it was determined that levobupivacaine and ropivacaine acted cytotoxic by activating apoptosis in SW480 colon cancer cells. It is thought that these data will lead to multidisciplinary studies to elucidate the antitumor effect mechanism of local anesthetics.

Keywords:. Colon cancer, levobupivacaine, ropivacaine, apoptosis

Öz

Amaç: Kolon kanseri, kanserler arasında en yaygın ölüm nedenleri arasında yer almakta olup, kolon kanseri tedavisinde rezeksiyon sonrası nüks ve metastazları önlemek için ağırlıklı olarak küratif cerrahi sonrası adjuvan kemoterapi uygulanmaktadır. Son yıllarda, lokal anestezikler kanser araştırmalarında giderek artan bir ilgi görmektedir. Bu çalışmada lokal anesteziklerden levobupivacaine ve ropivacaine'in SW480 kolon kanseri hücreleri üzerine proliferatif ve apoptotik etkilerinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Çalışmada, farklı konsantrasyonlarda ve sürelerde levobupivakain ve ropivakain, SW480 kolon kanseri hücre hattına uygulanarak MTT analizi ile proliferasyona etkisi belirlendi. Apoptotik etkinliğini belirlemek için ise TUNEL yöntemi kullanıldı. Sonuçlar istatistiksel olarak değerlendirildi.

Bulgular: Levobupivakain ve ropivakain' in doz ve zamana bağlı olarak SW480 kolon kanseri hücrelerinde hücre proliferasyonunu azalttığı belirlendi. Aynı zamanda kolon kanseri hücrelerinde apoptozu teşvik ettiği gözlendi.

Sonuç: Çalışma verilerine göre, levobupivakain ve ropivakain' in SW480 kolon kanseri hücrelerin de apoptozu aktive ederek sitotoksik etki gösterdiği tespit edildi. Bu verilerin lokal anesteziklerin antitümör etki mekanizmasını aydınlatmak için multidisipliner çalışmalara öncülük edeceği düşünülmektedir.

Anahtar kelimeler: Kolon kanseri, levobupivacaine, ropivacaine, apoptoz

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INTRODUCTION

Colon cancer ranks fourth among the world's leading tumors after lung, prostate, and breast cancer and is the second cause of cancer-related deaths[1]. As the world gets richer and more people to switch to Western diets and lifestyles, the incidence of colon cancer is likely to increase[2,3]. While advances in colon cancer treatment continue, chemotherapy is still limited by serious side effects and dose-limiting toxicities. Therefore, there is a need to develop new drugs that can effectively treat this type of cancer. Drug resistance is one of the biggest challenges in cancer treatment[4].

Recently, the reuse of old drugs has proven to be an appropriate strategy to create novel medications from this approved drugs[5]. Furthermore, these approved medications have already been tested on humans and found to have favorable profiles in terms of pharmacokinetics, pharmacodynamics, and safety, as well as undesirable/acceptable controlled harmful effects, which is a time-consuming procedure[6].

Inexpensive and economical, local anesthetics (LAs) are frequently used to treat pain using a variety of techniques, including intravenous administration, epidural or subarachnoid injection, local infiltration, and perioperative nerve block. Additionally, investigations conducted both in vivo and in vitro have demonstrated that LAs may have direct impacts on specific tumor cells (e.g., breast, lung, liver, and thyroid)[7,8]. Over the past decade, researchers have been working hard to discover novel local anesthetics with anti-cancer properties. They found that many anesthetics such as lidocaine, procaine, ropivacaine, bupivacaine, and levobupivacaine also have anticancer potential[8-11]. But until now, information about the direct interactions of LAs with cancer cells was still unclear.

Levobupivacaine is a long-acting local anesthetic that is commonly used in epidural, intrathecal, and ophthalmic anesthesia, as well as infiltration and nerve block [12]. Levobupivacaine has been found to have pharmacological antitumoral properties in primary and secondary cancer cells. It is also known to reduce cancer progression at specific concentrations in many types of cancer, such as prostate and breast cancer[13,14]. Ropivacaine is the most commanly used local anesthetic to relieve acute and chronic pain and cancer-related pain[12]. Some studies have shown that when used alone or in combination, ropivacaine may be effective as an anticancer drug at specific concentrations[11, 14-17].

The precise mechanisms of this activity of local anesthetics with significant anti-proliferative activity are not clear. The hypothesis of this study is to show how common local anesthetics levobupivacaine and ropivacaine affect the apoptotic death pathway on SW480 colon cancer cells in vitro.

Although there are many studies to determine the proliferative effect of local anesthetics, further studies are needed to determine their anticancer potential and to clarify their targets. This study aims to contribute to the literature by investigating the proliferative and apoptotic effects of two commonly used local anesthetics, levobupivacaine, and ropivacaine, on SW480 colon cancer cells.

MATERIALS AND METHODS

This study was carried out at Erciyes University Betül Ziya Eren Genome and Stem Cell Center (GENKOK), and GENKOK is one of Turkey's research centers engaged in research, development, and production in the field of health.

Our study is a cell culture study; neither humans nor experimental animals are used. Therefore, ethics committee approval is not required.

Cell culture

In the investigation, the colon cancer cell line SW480 (Manassas, USA: ATCC, CCL-228) was utilized. The cell line was grown in DMEM (Invitrogen, Carlsbad, CA, USA) media with 10% fetal bovine serum (Thermo Scientific), 2 mM L-glutamine, and 100 U/mL penicillin and streptomycin (Hyclone, ThermoScientific). Cells were kept at 37 °C and given 5% CO_2. Every three days, the cells' media were changed, and they were passaged when their density reached 90%.

Determination of drug-dose response

Levobupivacaine (5.0 mg/mL) was purchased from ABBOTT, and ropivacaine (7.5 mg/mL) was purchased from AstraZeneca. Following cell culture, levobupivacaine and ropivacaine were given in a range of doses (100 nM; 1 μM; 10 μM; 100 μM; 1 mM; 10 mM) to the cells. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test was applied to assess the effective doses of levobupivacaine and ropivacaine in the SW480 colon cancer cell line at 24,

48, and 72 hours. These doses and hours were used to calculate the IC50 value, which indicates the drug concentration required for 50% inhibition, and to assess the apoptotic effect of the drugs utilized in this test.

MTT test

Levobupivacaine and ropivacaine's effects on cell viability in the SW480 colon cell line were investigated using MTT measurements. Levobupivacaine and ropivacaine were added at concentrations ranging from 100 nM to 10 mM once the cell confluency reached 70% in two different 96-well plates (separately for levobupivacaine and ropivacaine). After 24, 48, and 72 hours, 10 µl of MTT was applied to each well of the cells, diluted with 0.5 mg/ml media. For one to four hours, 5% CO_2 was incubated at 37 °C. Following that, observations were performed at a wavelength of 570 nm [18]. All measurements were performed in triplicate.

Testing the effect on apoptosis

Levobupivacaine and ropivacaine's ability to induce apoptosis in the SW480 colon cancer cell line was examined using the TUNEL (terminal deoxynucleotide transferase-mediated 20-deoxyuridine 50-triphosphate nick endlabeling) technique.

On coverslips with cells seeded in accordance with the manufacturer's instructions, ApopTag® Fluorescein In Situ Apoptosis Detection Kit (Cat#S7110, EMD Millipore, Darmstadt, Germany) experiments were conducted. First, coverslips were twice-washed with PBS for five minutes and then treated with an equilibration buffer for 5 minutes. After applying the TUNEL mixture to the coverslip without washing it, it was incubated for 1 hour at 37°C in a humid and dark environment, and At the conclusion of the period, stop/wash buffer was applied and the coverslips were maintained at 37°C for 10 minutes. After washing the coverslips twice with phosphate-buffered saline (PBS) for 5 minutes, an anti-digoxigenin conjugate solution was applied to the coverslips and 30 minutes were kept at room temperature. PBS-washed coverslips were treated with DAPI for one minute, and the nuclei were stained. After the coverslips were washed again with PBS and sealed with the water-based sealer, the obtained preparations were examined under an Olympus BX51 fluorescent microscope [19].

Statistical analysis

The statistical analysis was carried out using the Graph-Pad Prism 9.4.1 program (San Diego, CA, USA). IC50 values of levobupivacaine and ropivacaine were calculated with Graph-Pad Prism 9.4.1 software. For each group, ten different microscopic fields at 400x original magnification were randomly obtained in order to identify apoptosis in the SW480 cell line. To evaluate the intensity of immunoreactivity, Image J software (Bethesda, USA) was utilized. One-way ANOVA and Bonferroni multiple comparison tests were used to evaluate the data. The results are presented as the mean standard deviation. Differences were deemed statistically significant at $p < 0.05$.

RESULTS

5×10^3 cells/well in a 96-well plate after incubation for 24 hours were administered various dosages of levobupivacaine and ropivacaine (100 nM–10 mM) for 24, 48, and 72 hours, respectively. SW480 cells showed mortality depending on the dose and the passage of time. As shown in figure 1, the IC50 values of levobupivacaine and ropivacaine were 844.6 µM and 1305 µM for 24 hours, respectively.

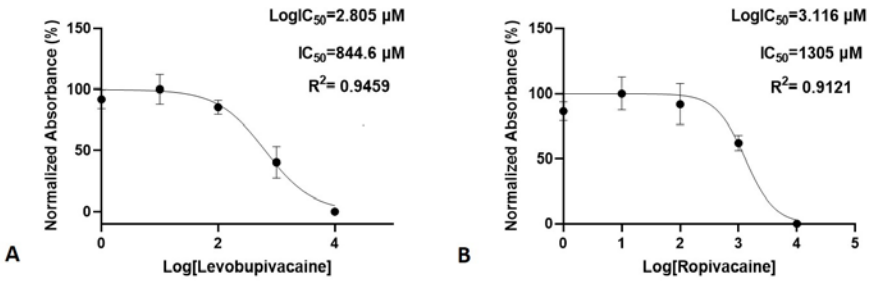

Figure 1. Dose response curves for levobupivacaine (A) and ropivacaine (B) (24 hours).

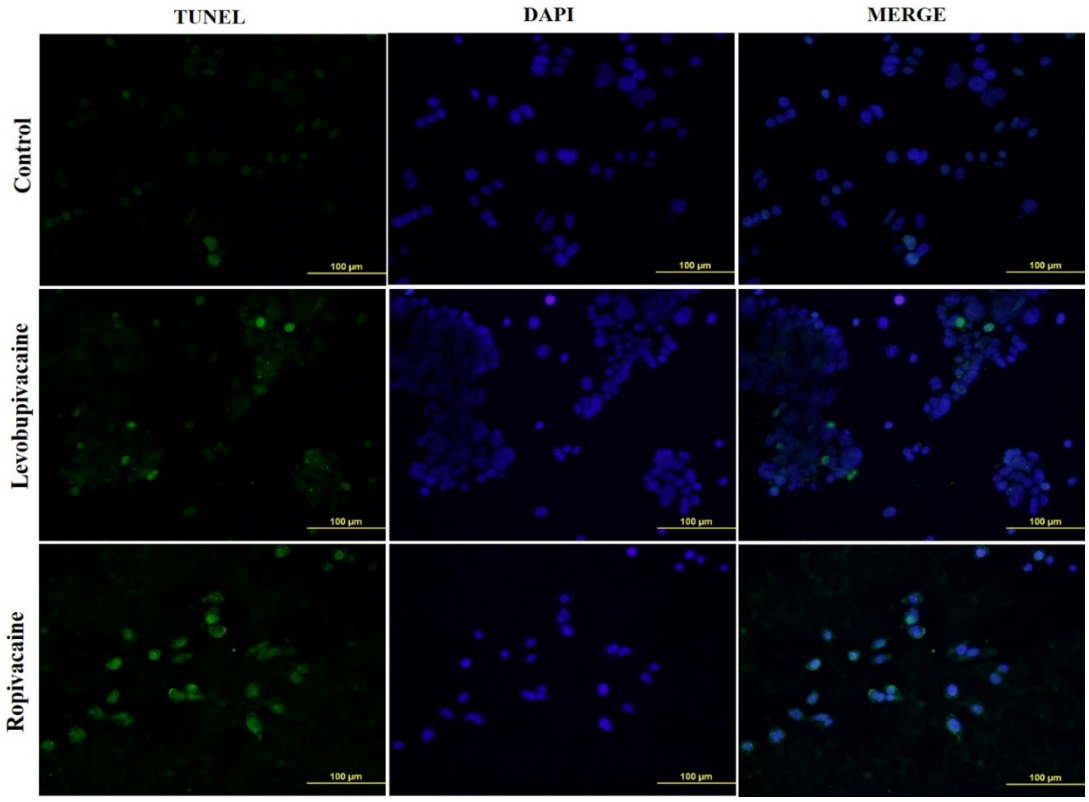

Figure 2. SW480 cell line TUNEL images. Under a fluorescence microscope, the cells' released apoptotic bodies appeared as green reflections (400x).

TUNEL-positive cells were shown to be stained green under the fluorescence microscope (Figure 2).There was a noticeable increase in TUNEL+ apoptotic cells in SW480 cells when comparing the levobupivacaine group to the control group (p=0.0046). Additionally, the ropivacaine group showed a statistically significant increase in TUNEL+ apoptotic cells when compared to the control group (p<0.0001). The results showed that the ropivacaine group had a statistically significantly higher number of TUNEL+ apoptotic cells than the levobupivacaine group (p=0.0113) (Table 1).

Table 1. Results of statistical analysis of TUNEL expression of levobupivacaine and ropivacaine on SW480 colon cancer cell line.

	Groups			
TUNEL	**Control**	**Levobupivacaine**	**Ropivacaine**	**p**
	(1.20±0.08)a	(1.77±0.30)b	(2.28±0.54)c	<0.0001

The mean ± standard deviation is used to express data, refers to the importance of the group differences. The same lowercase letters on the same line indicate similarity between groups, and different lowercase letters show differences between groups. For significance, a P value of < 0.05 was employed.

DISCUSSION

Despite significant advances in cancer treatment, there is still a need for new therapeutic drugs with anticancer properties to overcome many important challenges[20]. Local anesthetics used during surgery have been linked to lower rates of recurrence and metastasis formation, and thus a higher survival rate[21]. In recent years, local anesthetics have received increasing attention in cancer research. More in-depth research is required to clarify the particular uses and targets of local anesthetics' anticancer potential[5,6].

Levobupivacaine is one of the long-acting local anesthetics and is widely used[12]. Multiple mechanisms have been associated with the reduction of cell proliferation induced by levobupivacaine. Kwakye et al. explored the effect of levobupivacaine on MCF-7 and MDA-MB-231 breast cancer cells in their study. They observed that levobupivacaine reduced Bcl-2 and increased Bax in breast cancer cells, leading the cells to apoptosis and inhibiting proliferation[14]. According to Castelli et al. treatment with 50 μM levobupivacaine resulted in 50% death of the MDA-MB-231 cell line after 24 hours of treatment. Moreover, this LA promoted the inactivation of survival pathways such as PI3K/Akt/GS3K/β-catenin, which contributes to cell death via apoptosis[22]. Mao et al. demonstrated in vitro and in vivo that the local anesthetic levobupivacaine induces ferroptosis and suppresses cell proliferation in gastric cancer[23]. Jose et al. reported that levobupivacaine has a potent and specific antiproliferative effect on human prostate cancer cells compared to non-cancer homologs. It was also discovered that combining levobupivacaine with an autophagy blocker activates autophagy in prostate cancer cells and increases cytotoxicity[13]. Li T et al. discovered that levobupivacaine inhibits cell migration and proliferation in the Caco-2 colon cancer cell line[10]. In addition to the literature, this study evaluated the apoptotic effect as well as the proliferative effect of levobupivacaine on the SW480 colon cancer cell line.

Ropivacaine is a new local anesthetic agent with a long half-life[24]. Various molecular mechanisms have been proposed for the cytotoxic effects of ropivacaine in cancer cells. Zhau L. et al. showed that ropivacaine inhibits proliferation and induces apoptosis in both MCF-7 and MDA-MB-231 breast cancer cells[25]. Ropivacaine on breast cancer cells, at doses ranging from 0.05 to 10 mM, promoted cell death and selectively inhibited cell proliferation and migration. In non-tumor cells, MCF-10A, ropivacaine appears to have no effect, resulting in LA suggesting that it affects only cancer cells[26]. In hepatocellular carcinoma, ropivacaine was found to cause cell death by regulating caspase-3 activation[27]. Ropivacaine, lidocaine, and the combination all had cytotoxic effects on human melanoma cell lines (A375 and Hs294T), depending on the duration and concentration. The combination, however, was less cytotoxic than ropivacaine, lidocaine, and lidocaine + ropivacaine[28]. Siekmann et al. found that the colon cancer cell lines SW480 and SW620 showed an anti-proliferative effect when exposed to high concentrations of ropivacaine for a long time[29]. In this study, only the proliferative effect of the drug was evaluated, and the pathway by which it suppressed cell viability was not investigated. It has been reported that analgesics such as ropivacaine and bupivacaine can induce the intrinsic apoptosis pathway in rabbit annulus fibrosus cells in vitro[30]. Our results support prior research's conclusions that ropivacaine causes colon cancer cells to undergo apoptosis, which reduces cell viability. As a limitation of the study, it can be made more important by elucidating the similarities and differences between the mechanistic effects of local anesthetics on different cancer cell lines.

Levobupivacaine and ropivacaine, which are frequently used for local anesthesia, have changed how quickly colon cancer cells multiply. When the effects of levobupivacaine and ropivacaine on SW480 colon cancer cells were examined, ropivacaine was found to cause more cell apoptosis. This demonstrates that the treatment of colon cancer cells with ropivacaine may be more successful. The effectiveness of levobupivacaine and ropivacaine in the treatment of colon cancer can be studied in vivo in the future.

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