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ORIGINAL ARTICLE

Ropivacaine via nuclear factor kappa B signalling modulates CD62E expression and diminishes tumour cell arrest

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Abstract

Background The issue whether anaesthesia has an impact on the prognosis of carcinoma has been widely discussed and remains debated. Ropivacaine has been widely used in perioperative period as a long acting local anesthetic. An early event during recurrence or metastasis of carcinoma is the adhesion of circulating tumour cells (CTCs) to endothelial cells (ECs) through binding adhesion molecules that are up-regulated on inflamed endothelium during the perioperative period or other periods. This study was to explore the impact of ropivacaine on the adhesion of tumour cells, providing evidences of its influence on the prognosis of carcinoma.

Materials and methods Human umbilical vein endothelial cells (HUVECs) were pre-treated with ropivacaine (10^{-7}–10^{-5} M; 30 min) prior to treatment with tumour necrosis factor alpha (TNFα) (10 ng ml^{-1}; 1, 4 and 8 h). Intercellular adhesion molecule-1 (ICAM-1), endothelial-selectin (CD62E) and vascular cell adhesion molecule-1 (VCAM-1) mRNA levels were detected via quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR). To clarify the underlying action mechanism, p65, p-p65, IκBα, p-IκBα, IKKα/β and p-IKKα/β protein levels were evaluated via western blotting. Cell viability and tumour cell adhesion assays were also assessed.

Results The clinically usage concentration of ropivacaine (10^{-6} M) produced a significant decrease in CD62E expression compared with that produced by TNFα only ($p < 0.001$). Moreover, adhesion assays showed that ropivacaine effectively inhibited the adhesion of hepatoma cells ($p < 0.01$), human colon cancer cells ($p < 0.01$) and human leukemic monocyte ($p < 0.01$). Western blot results showed that pre-treatment with ropivacaine inhibited the phosphorylation of p65 ($p < 0.05$), IκBα ($p < 0.001$) and IKKα/β ($p < 0.01$).

Conclusions Ropivacaine decreased the adhesion of tumour cells. Ropivacaine modulated CD62E expression by inhibiting the activation of NF-κB. These results might provide new insight into the issue whether anaesthesia has an impact on the prognosis of carcinoma.

Keywords Ropivacaine · Cell adhesion · Adhesion molecules · CD62E · Tumour cell · Nf-kappa B signalling.

Introduction

Cancer is one of the most life-threatening diseases [1]. Surgery is the most effective therapy for solid carcinomas, and anaesthesia is necessary for this treatment. Compared

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with general anaesthesia, regional anaesthesia (RA) is proved to bring better outcomes [2–5]. And ropivacaine has been widely used for regional anaesthesia as a long acting local anesthetic.

Surgery is the most effective therapy for solid carcinomas, meanwhile surgery increases the number of circulating tumour cells (CTCs) [6, 7], suppresses cell-mediated immunity [8] and induces an acute inflammatory response [8, 9].

CTC is one of the key factors affecting the prognosis of tumors [10, 11]. CTCs arrest of ECs is an early event in tumour metastasis or recurrence, and this event depends on a variety of ligands and receptors, including selectins, integrins, cadherins, CD44 and immunoglobulin superfamily receptors on cancer cells and ECs [12]. CD62E (E-selectin) seems to be important for tumour metastasis [9, 12–16]. Hyperpemeable foci is preferentially home to CTCs via CD62E, an endothelial adhesion molecule [17]. The ligands CD62E, sLe^X, sLe^A and CD44 are expressed on the surface of tumour cells such as hepatoma cells [18, 19], human colon cancer cells [18] and human leukemic monocyte [20]. CD62E is not normally expressed on quiescent ECs, but is induced by inflammatory cytokines such as TNFα. The expression of CD62E is induced through NF-κB activation [21, 22]. In brief, binding of the inherently trimeric TNFα to TNFR1 (tumour necrosis factor receptor I), recruits tumour necrosis factor receptor-associated death domain protein (TRADD), tumour necrosis factor receptor-associated factor 2/5 (TRAF2/5) and receptor-interacting protein kinase 1 (RIP1), and leads to the activation of IKK complexes. Activation of IKK complexes invariably leads to IκB phosphorylation, IκB degradation and p65 (NF-κB) phosphorylation. The phosphorylated p65, which leads to NF-κB DNA binding activity, translocates from cytoplasm to nucleus. Then phosphorylated p65 induces NF-κB-dependent gene expression [23, 24]. The CD62E gene contains three NF-Kappa B binding sites in the 5′-flanking promoter region [21, 22].

Based on laboratory studies and retrospective clinical studies, anaesthesia probably has an impact on the prognosis of carcinoma, but it has not yet been confirmed. Regional anaesthesia reduces the demand of opioids, preserves the immune system and attenuates the surgical stress response [8, 25]. Ropivacaine is a widely-used local anaesthetic for regional anaesthesia, and its anti-inflammatory actions on endothelial cells (ECs) are well known [26, 27], but there're few researches focusing on the impact of local anesthetics on tumor cell adhesion.

So during the perioperative period, increasing number of CTCs are attached to ECs via adhesion molecules in inflamed vessels. Ropivacaine is used during the perioperative period, and its anti-inflammatory action in endothelial cells (ECs) is well known [26, 27], but whether ropivacaine modulates adhesion molecule expression and inhibits the adhesion of tumour cells and its associated mechanism in ECs have not yet been fully determined.

In this work, we first demonstrated that the clinically usage concentration of ropivacaine inhibited CD62E expression and the adhesion of tumour cells. Moreover, the cascade activation of NF-κB signalling was inhibited by ropivacaine.

Materials and methods

Reagents

The reagents used included BAY-117082 and JSH-23 (Selleck, Shanghai, China), Calcein-AM (Santa Cruz, CA, US), 1% ropivacaine (Naropin®, APP Pharmaceuticals, Schaumburg) and human recombinant TNFα (PeproTech, NJ, USA).

Cell culture

Human umbilical vein endothelial cells (HUVECs) were separated from human umbilical cord veins according to previous protocols, with minor modifications [28]. This study was approved by the ethics committee of Sun Yat-sen Memorial Hospital of Sun Yat-sen University (Guangzhou, People's Republic of China). Human umbilical veins were perfused with phosphate buffered saline (PBS) and then treated with 0.1% type I collagenase (20 ml, 12 min, 37 °C; Life Technologies, Shanghai, China). After the removal of collagenase, the cells were cultured in flasks in Endothelial Cell Medium (ECM; ScienCell, Shanghai, China). HUVECs at fewer than 5 passages were used. HepG2 (human hepatoma cells), HT-29 (human colon cancer cells) and THP-1 cells (human leukemic monocyte) were obtained from the Chinese Academy of Sciences Cell Bank of Type Culture Collection. HepG2 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Guangzhou, China), and THP-1 and HT-29 cells were cultured in RPMI 1640 medium (Gibco, Guangzhou, China). DMEM and RPMI 1640 media were supplemented with 10% foetal bovine serum (Gibco, Guangzhou, China), penicillin (100 units ml^{-1}; Gibco, Guangzhou, China) and streptomycin (100 μg ml^{-1}; Gibco, Guangzhou, China), and then all cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air.

Cell viability

The effects of ropivacaine on HUVEC viability were evaluated with 3-(4,5-dimethyl-2-thiazyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT; Biosharp, Hefei, China). In brief, 3,000 cells were seeded in 96-well culture plates and cultured overnight, followed by the addition of ropivacaine for 48 h. Then, the cells were incubated with 20 μl MTT (5

mg ml^{-1}, 4 h, 37 °C), the medium was discarded, and 150 µl dimethyl sulphoxide (DMSO; Sigma Chemical, St. Louis, USA) was used to resolve the crystals. The staining intensity in DMSO was measured at 570 nm. Cytotoxicity was measured by the number of surviving cells in each treatment expressed as the percentage of untreated cells. The values were expressed as the mean ± standard deviation (SD) of four separate experiments, each performed in triplicate.

Adhesion assay

The effects of ropivacaine on cell adhesion were examined by a fluorescence-based analysis. HUVECs grown to confluence in 24-well plates were pre-incubated with ropivacaine for 30 min prior to TNFα (10 ng ml^{-1}, 4 h). The culture medium containing ropivacaine was removed and then Calcein-AM-labelled HepG2, HT-29 or THP-1 cells were added and cultured with the HUVEC monolayers. After incubation for 30 min, non-adhered cells were washed out with PBS. Five images per well were randomly taken using a fluorescence microscope (Nikon ECLIPSE Ti, Japan). The inhibition of adhesion was calculated as previously described [29]: relative adhesion = (the number of adhered cells in treated samples/the number of adhered cells in the TNFα group) × 100%.

RNA isolation and quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR)

Total RNA was extracted using TRIzol reagent (Invitrogen, California, USA). A PrimeScript™ RT reagent kit (Takara, Shanghai, China) was used to synthesize cDNA. qRT-PCR was performed on a LightCycler 480 system (Roche, Mannheim, Germany) using 2 × SYBR Green qPCR Master Mix (Biotool, Shanghai, China). Different primer pairs were used for each reaction. CD62E: forward: 5′-TCCCTC CTGACATTAGCACC-3′, reverse: 5′-GTGTATCCCTCT AGTTCCCCAG-3′; ICAM-1: forward: 5′-GGCAGAGTA CGCAAACACTT-3′, reverse: 5′-GGCTGTAGCTCCCCG TTAG-3′;VCAM-1: forward: 5′-CGAACCCAAACAAAG GCAGAG-3′, reverse: 5′-CCTGGCTCAAGCATGTCA TATTC-3′; GAPDH: forward: 5′-GAAGGTGAAGGTCGG AGTCAACG-3′, reverse: 5′-TGCCATGGGTGGAATCAT ATTGG-3’.

Western blotting

HUVECs were harvested and lysed using RIPA buffer (Beyotime, Haimen, China). SDS-PAGE (8% or 10%) was used to separate denatured protein, and polyvinylidene fluoride membranes (PVDF; 0.22 µm, Bio-Rad, California, USA) were used as the transfer medium. After completing the protein transfer, the PVDF membrane was blocked in 5% (w/v) non-fat milk in TBST and incubated overnight at 4 °C with the primary antibody, GAPDH (1:2000, Product # sc-47724, Santa Cruz Biotechnology, Shanghai, China), IKKα (1:1000, Product # 11930, Cell Signaling Technology, Shanghai, China), IKKβ (1:1000, Product # 8943, Cell Signaling Technology, Shanghai, China), Phospho-IKKα/β (1:500, Product # 2687, Cell Signaling Technology, Shanghai, China), P65 (1:1000, Product # 8242, Cell Signaling Technology, Shanghai, China), Phospho-P65 (1:1000, Product # 3033, Cell Signaling Technology, Shanghai, China), IκBα(1:1000, Product # 4814, Cell Signaling Technology, Shanghai, China), Phospho-IκB-α (1:1000, Product # 2859, Cell Signaling Technology, Shanghai, China). The blots were detected using a horseradish peroxidase-linked goat anti-rabbit/mouse secondary antibody (1:20,000, Product # sc-2004/sc-2005 Santa Cruz Biotechnology, Shanghai, China) and visualized with SuperSignal West Dura (Thermo, Rockford, IL). All western blots were repeated 3 times.

Statistical analyses

All data are expressed as the means ± SDs. Mean comparisons were performed using SPSS 16 via one-way ANOVA with Bonferroni post hoc corrections for multiple comparisons or two-tailed Student's t test, as appropriate; $p < 0.05$ was deemed statistically significant.

Results

Effects of ropivacaine on HUVECs viability

The cytotoxic effects of local anaesthetics have been widely discussed. The concentrations of ropivacaine that were effective (10^{-8}–10^{-6} M) are clinically relevant [30]. Compared with HUVECs incubated with vehicle, ropivacaine [10^{-8} M (98.20 ± 7.08%), 10^{-7} M (97.84 ± 3.13%), 10^{-6} M (92.48 ± 3.79%), and 10^{-5} M (90.96 ± 7.41%), $p > 0.05$, Fig. 1] did not affect cell viability. Ropivacaine [10^{-4}M (57.38 ± 6.57%) and 10^{-3} M (42.56 ± 3.69%), $p < 0.0001$, Fig. 1] significantly inhibited HUVECs viability. These data suggested that clinically usage concentrations of ropivacaine did not reduce cell viability.

Ropivacaine efficiently inhibited the adhesion of hepatoma cells, human colon cancer cells and human leukemic monocyte to HUVECs.

CTCs' adhering to ECs is an early event in tumour metastasis and recurrence [12]. Our results revealed that TNFα significantly increased the adhesion of HT-29, HepG2 and THP-1 cells to HUVECs. Compared with HUVECs incubated with TNFα alone, the adhesion of HepG2 (53.78 ± 16.97%, $p < 0.001$, Fig. 2a), HT-29 (61.38 ± 14.75%,

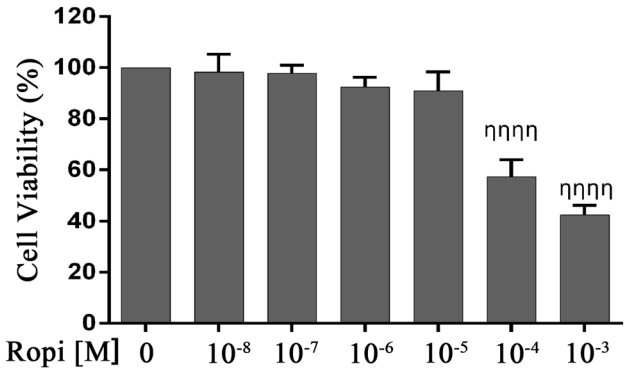

Fig. 1 Effects of ropivacaine on HUVEC viability. HUVECs were pre-incubated with ropivacaine at the indicated concentration for 48 h. The data are expressed as the percentage of control cells; mean ± SD of four separate experiments, each performed in quadruplicate. $^{\eta\eta\eta\eta}p < 0.0001$ compared with control group. Using one-way ANOVA. *Ropi* ropivacaine

$p < 0.001$, Fig. 2b) and THP-1 (45.74 ± 18.42%, $p < 0.001$, Fig. 2c) cells was significantly decreased by pre-treating with ropivacaine.

Inhibition of CD62E expression by ropivacaine

The adhesion of CTCs to ECs depends on cell adhesion molecules that are up-regulated on inflamed endothelium such as ICAM-1, CD62E and VCAM-1 [12, 31]. In our study, compared with HUVECs treated with vehicle, TNFα induced up-regulation of CD62E (1087.21 ± 84.62, $p < 0.001$, Fig. 3a), ICAM-1 (489.63 ± 32.82, $p > 0.05$, Fig. 3b) and VCAM-1 (311.05 ± 72.08, $p < 0.001$, Fig. 3c) in HUVECs. Compared with HUVECs treated with TNFα alone, HUVECs pre-treated with ropivacaine (10^{-6} M (677.03 ± 28.90), $p < 0.001$, Fig. 3a) exhibited significantly-decreased CD62E expression. Moreover, neither the expression of ICAM-1 (489.44 ± 75.60, $p > 0.05$, Fig. 3b) nor VCAM-1 (283.29 ± 21.54, $p > 0.05$, Fig. 3c) was affected by ropivacaine. The inhibition effect of ropivacaine on CD62E expression was concentration-dependent manner (Fig. 4a). At different time courses, ropivacaine showed its inhibition effect on CD62E expression (Fig. 4b).

Ropivacaine inhibited the expression of CD62E via NF-κB signalling

TNFα induces CD62E expression, which is well known to depend on NF-kappa B pathway activation [21, 22]. In our study, compared with HUVECs treated with vehicle, TNFα induced up-regulation of CD62E (1368.46 ± 232.86, $p < 0.001$, Fig. 5) in HUVECs. The NF-κB pathway inhibitors BAY-117082 [(5 μM, 13.93 ± 4.52, $p < 0.001$) (2 μM, 105.69 ± 16.51, $p < 0.001$) (1 μM, 755.11 ± 130.94, $p < 0.001$), Fig. 5] and JSH-23 (50 μM, 332.49 ± 72.32, $p < 0.001$, Fig. 5) significantly inhibited CD62E expression compared with TNFα alone. HUVECs that were pre-incubated with either BAY-117082 (209.50 ± 11.43, $p > 0.05$, Fig. 5) or JSH-23 (301.26 ± 73.40, $p > 0.05$, Fig. 5) following treatment with ropivacaine exhibited no significant decrease in CD62E expression compared with those pre-incubated with an inhibitor alone. These results indicated that, after blocking the NF-κB pathway by NF-κB signalling inhibitors before ropivacaine treatment, ropivacaine did not efficiently inhibit CD62E expression. Furthermore, these findings provided indirect evidences that NF-κB signalling was involved in the modulation of CD62E expression by ropivacaine.

Pre-treatment with ropivacaine inhibited activation of the NF-κB signalling cascade

To delineate the mechanism by which ropivacaine inhibited CD62E expression, we examined the activation of the NF-κB signalling cascade. The phosphorylation of p65 was reduced to 62.88 ± 7.34% ($p < 0.05$, Fig. 6), the phosphorylation of IκBα observed in response to TNFα alone was reduced to 36.91 ± 5.39%, and the total IκBα observed in response to TNFα alone was increased to 183.04 ± 12.89% in the presence of 10^{-6} M ropivacaine ($p < 0.01$, Fig. 6) after the pre-treatment of 10^{-6} M ropivacaine.

Phosphorylation of IKKα/β was inhibited by ropivacaine

Further, we examined the phosphorylation of IKKα/β, which leads to the phosphorylation of p65 and IκBα. We observed that TNFα significantly increased phosphorylation of IKKα/β, and it was reduced to 50.55 ± 11.74% by 10^{-6} M ropivacaine ($p < 0.01$, Fig. 7).

Discussion

The present results showed that the clinically usage concentration of ropivacaine (10^{-6} M) significantly inhibited the expression of CD62E, whereas ropivacaine (10^{-6} M) did not inhibited the expression of ICAM-1 and VCAM-1. Pre-treatment with ropivacaine significantly inhibited the adhesion of tumour cells to TNFα-activated monolayer HUVECs. The data also suggested that the prophylactic inhibitory effects of ropivacaine occurred via the inhibition of NF-κB pathways. Therefore, the inhibition of adhesion molecules showed in this study suggested that ropivacaine could be beneficial for the treatment of carcinoma patients who would require surgery as an intervention.

Numerous retrospective studies have suggested that regional anaesthesia (paravertebral [2] and epidural block

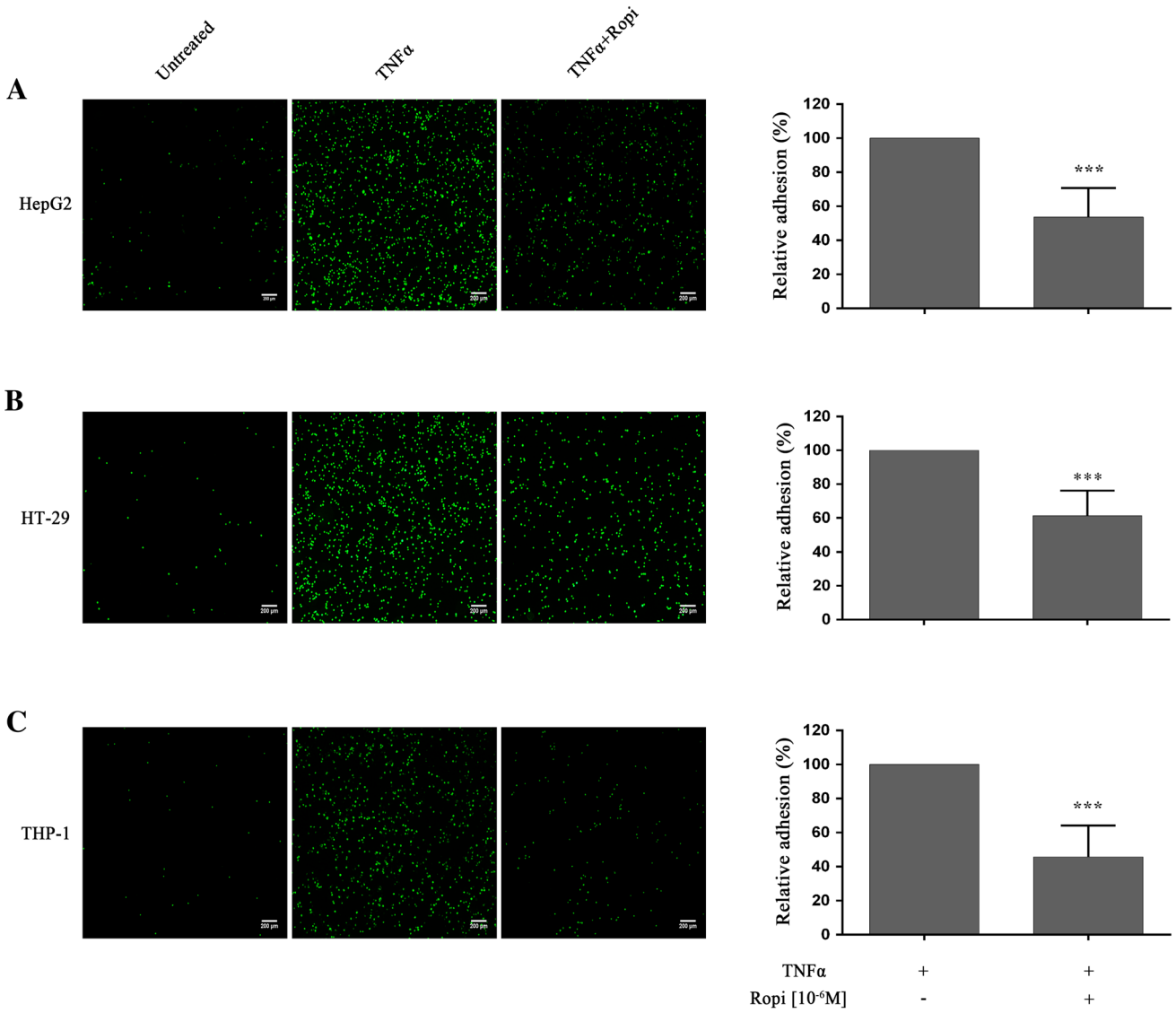

Fig. 2 Ropivacaine efficiently inhibited the adhesion of hepatoma cells, human colon cancer cells and human leukemic monocyte to HUVECs. Calcein-AM-labelled **a** HepG2 cells (green), **b** HT-29 cells (green) and **c** THP-1 cells (green) were added to HUVEC monolayers stimulated by TNFα (10 ng ml^{-1}, 4 h) after pre-incubation with ropivacaine (10^{-6} M, 30 min). Representative fluorescence microscopy images showed that **a** HepG2 cells (green), **b** HT-29 cells (green) and **c** THP-1 cells (green) adhered to the HUVECs. Quantification of HepG2, HT-29 and THP-1 cells adhered to the HUVEC monolayers in the presence of ropivacaine. Scale bars = 200 μm. The data are expressed as the percentage of TNFα group; mean ± SD of five separate experiments, each performed in quintuplicate. ***$p < 0.001$ compared with TNFα group. Using Student's *t* test. *Ropi* ropivacaine

[3–5]) are associated with a decreased risk of recurrence or metastasis of multiple carcinomas, including breast [2], prostate [3], colon and rectum [5] cancers. Previous studies regarding how anaesthesia affects carcinoma prognosis have focused on the consumption of opiates [8], the influence of anaesthesia on the immune system [8], the relief of pain and the implications of anaesthetics on tumour cell characteristics [25, 32, 33]. However, surgery not only causes an increasing number of CTCs [6], which is an independent prognostic marker for cancer recurrence, but also induces inflammatory responses [8, 9]. Furthermore, surgery increases the plasma levels of pro-inflammatory cytokines [8, 9]. These pro-inflammatory cytokines might result in increasing vascular permeability and the expression of cell adhesion molecules [34, 35].

Local anaesthetics block peripheral or spinal nerve conduction by blocking voltage-gated sodium channels, thus producing analgesic effects. Local anaesthetics not only exert an analgesic effect but also other pharmacological actions, such as anti-inflammatory effects [36, 37], anti-carcinoma effects [32] and vascular protection [26]. Ropivacaine is widely used in epidural anaesthesia and patient-controlled

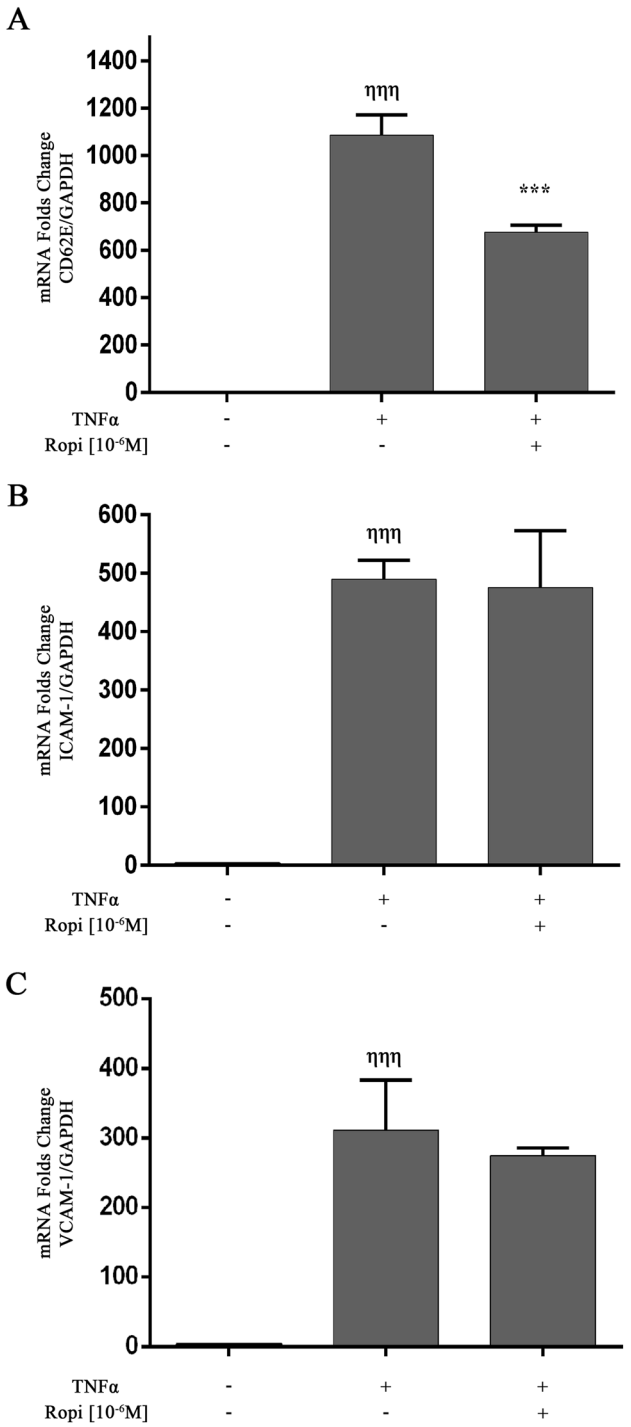

Fig. 3 Inhibition of CD62E expression by ropivacaine. HUVECs were pre-incubated with ropivacaine (10^{-6} M) for 30 min prior to TNFα stimulation (10 ng ml^{-1}, 4 h). CD62E (**a**), ICAM-1 (**b**) and VCAM-1 (**c**) were assessed via qRT-PCR and normalized to GAPDH. Bars represent the mean ± standard error of the mean (SEM; $n=3$). $^{ηηη}p<0.001$ compared with compared with control group, $^{*}p<0.05$, ** $p<0.01$ and *** $p<0.001$ compared with TNFα group. Using one-way ANOVA. *Ropi* ropivacaine

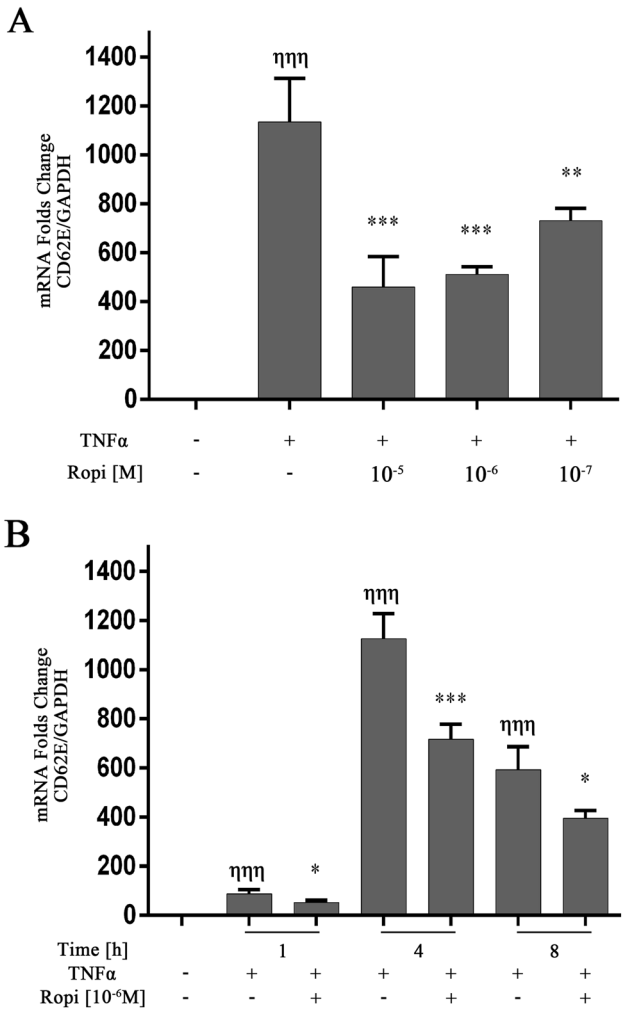

Fig. 4 The inhibition effects of ropivacaine on CD62E expression was concentration dependent manner and at different time course. **a** HUVECs were pre-incubated with different concentrations of ropivacaine for 30 min prior to TNFα stimulation (10 ng ml^{-1}, 4 h); mRNA was assessed via qRT-PCR and normalized to GAPDH. **b** HUVECs were pre-incubated with ropivacaine (10^{-6} M) for 30 min prior to TNFα stimulation (10 ng ml^{-1}) for the indicated times. CD62E mRNA expression was assessed via qRT-PCR and normalized to GAPDH. Bars represent the mean ± standard error of the mean (SEM; $n=3$). $^{ηηη}p<0.001$ compared with compared with control group, $^{*}p<0.05$, ** $p<0.01$ and *** $p<0.001$ compared with TNFα group. Using one-way ANOVA. *Ropi* ropivacaine

epidural analgesia (PCEA). Typically-used medication formula of PCEA includes ropivacaine and morphine during the post-operative period in China. The concentration of ropivacaine (10^{-6} M) that was effective in this study is clinically relevant. The maximum plasma concentration (C_{max}) of ropivacaine reaches 5.3×10^{-6} M during continuous epidural infusions of 0.2% ropivacaine for 48 h after major abdominal, urological, or gynaecological surgerys [30], similar concentrations of ropivacaine also have been reported [38, 39]. And in the transversus abdominis plane block [40], the C_{max}

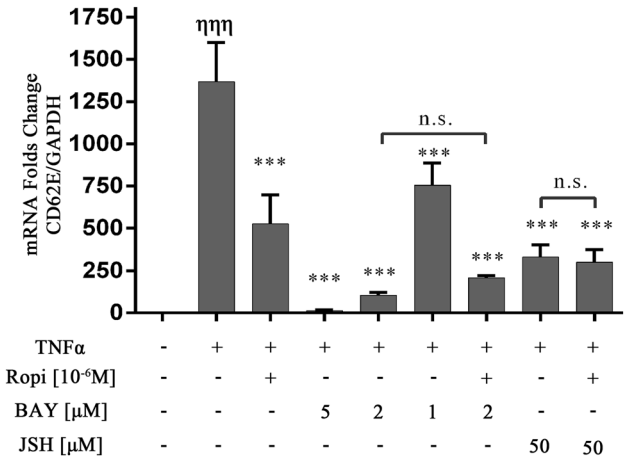

Fig. 5 NF-κB signalling was involved in the modulation of CD62E expression by ropivacaine. HUVECs were pre-incubated with different concentrations of BAY117082 or JSH-23 for 1 h prior to TNFα (10 ng ml^{-1}, 4 h) or pre-incubated with BAY117082 (2 μM) or JSH-23 (50 μM) for 30 min before ropivacaine was added (10^{-6} M) and co-incubated for 30 min prior to the addition of TNFα (10 ng ml^{-1}, 4 h). CD62E mRNA levels were assessed via qRT-PCR and normalized to GAPDH; Bars represent the mean ± SEM ($n = 3$). $^{ηηη}p < 0.001$ compared with compared with control group, $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with TNFα group. *Ropi* ropivacaine. Using one-way ANOVA. *Ropi* ropivacaine, *BAY* BAY-117082, *JSH* JSH23

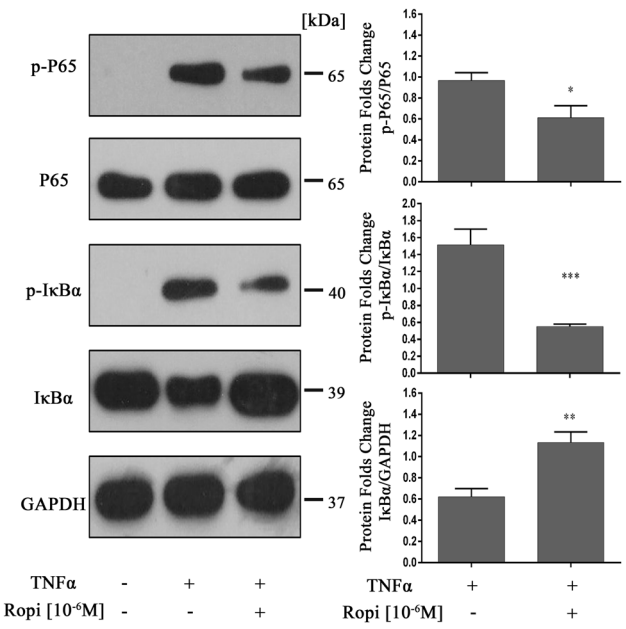

Fig. 6 Ropivacaine inhibited NF-κB signalling activation. Western blot experiments showing the effect of the ropivacaine (10^{-6} M) treatment of HUVECs for 30 min prior to TNFα stimulation (10 ng ml^{-1}, 1 h) on the phosphorylation of the IκBα and p65 proteins. The blot was probed for the different proteins, and GAPDH was used as a control. A representative example of the experiments is depicted. Quantitative analysis of the densitometry of the western blots showing the ratios of p-P65 to P65, p-IκBα to total IκBα and total IκBα to GAPDH. Bars represent the mean ± SEM ($n = 3$). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with TNFα group. Using Student's *t* test. *Ropi* ropivacaine

of ropivacaine reaches $(5.5 \pm 1.2) \times 10^{-6}$ M, and the time to reach C_{max} (t_{max}) is (0.44 ± 0.36) h after injection of 150 mg 0.75% ropivacaine. In continuous interscalene analgesia [41], the C_{max} of ropivacaine reaches $(4.3 \pm 1.6) \times 10^{-6}$ M, and the t_{max} is (43 ± 17) h after the injection of 9 ml h^{-1} 0.2% ropivacaine for 48 h. In continuous lumbar plexus blockade [42], the C_{max} of ropivacaine reaches $(8.0 \pm 4.5) \times 10^{-6}$ M after the infusion of 12 ml h^{-1} 0.2% ropivacaine for 48 h.

It has been reported that ropivacaine protects the endothelial barrier against TNFα-induced disruption [27] and inhibits neutrophil adhesion to ECs [26]. Meanwhile, the adhesion of CTCs to ECs and the adhesion of neutrophils to ECs share similar mechanisms and cell-surface adhesion molecules [12]. In this study, we demonstrated that ropivacaine significantly diminished the adhesion of HepG2, HT-29 and THP-1 cells to HUVECs (Fig. 2).

Various studies have reported that adhesion molecules promote carcinoma recurrence or metastasis. For example, CD62E up-regulation on ECs is correlated with carcinoma cell adhesion [17]. In addition, increased CD62E promotes liver metastasis [15, 43, 44] after hepatic ischaemia–reperfusion injury. We found that ropivacaine inhibited CD62E expression but not ICAM-1 and VCAM-1 expression (Figs. 3, 4), which is in accord with previous findings that ropivacaine inhibited the phosphorylation of ICAM-1, but not total ICAM-1, via the Src pathway [26].

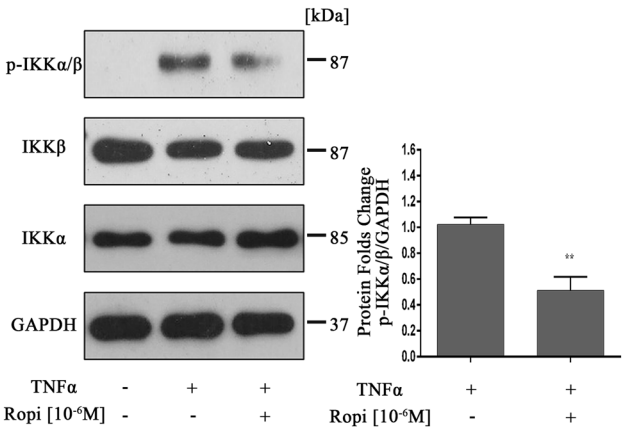

Fig. 7 Phosphorylation of IKKα/β was inhibited by ropivacaine. Cells were pre-incubated with ropivacaine for 30 min prior to TNFα stimulation (10 ng ml^{-1}, 1 h). A representative example of the experiments is depicted. Quantitative analysis of the densitometry of the western blots showing the ratio of p-IKKα/β to GAPDH. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with TNFα group. Using Student's *t* test. *Ropi* ropivacaine

The CD62E gene contains three NF-Kappa B binding sites in the 5′-flanking promoter region, and the expression of CD62E is induced by NF-κB activation [21, 22]. In our study, the NF-κB signalling inhibitors JSH-23 and BAY-117082 significantly inhibited CD62E expression. Ropivacaine did not inhibit CD62E expression after pre-incubation with JSH-23 or BAY-117082 compared with pre-incubation with JSH-23 or BAY-117082 prior to TNFα; this finding indirectly demonstrated that NF-κB signalling was involved in inhibition of CD62E expression by ropivacaine. (Fig. 5).

It is reported that ropivacaine inhibits TNFα-induced phosphorylation of IκBα and p65 translocation to the nucleus in mesenchymal stem cells [45]. Our results showed that the protein level of IκBα was significantly increased by ropivacaine; in addition, phosphorylation of p65 and IκBα was attenuated. And we found that the phosphorylation of IKKα/β, which was an upstream of p65 and IκBα, was significantly increased by TNFα stimulation and inhibited by ropivacaine (Fig. 7). However, ropivacaine reportedly inhibits Akt signalling by blocking the recruitment of p85 to TNF-R1 [26]. TNF-R1, as a TNFα receptor, recruits tumour necrosis factor receptor-associated death domain protein (TRADD), tumour necrosis factor receptor-associated factor 2/5 (TRAF2/5), UbcH5 and cIAP1 and subsequently forms ubiquitin chains to receptor-interacting protein kinase 1 (RIP1) [46, 47].TAB/TAK1 and IKK complexes bind to these ubiquitins, followed by the activation of TAK1 and phosphorylation and activation of IKKβ [47, 48]. Above all, we believed that the primary target of ropivacaine was not IKKα/β, but rather that it was most likely upstream of IKKα/β. Thus, an IKK kinase assay should be additionally performed, and the primary target of ropivacaine in NF-κB pathway inhibition should be explored.

Several important limitations of this work: all of the results were obtained from in vitro experiments and in vivo experiments were not included. The primary target of ropivacaine in NF-κB pathway inhibition has not yet been explored.

In conclusion, We demonstrated that ropivacaine inhibited the adhesion of tumour cells to endothelial cells. We also found that ropivacaine inhibited the expression of CD62E expression by inhibiting the activation of NF-κB pathway. Thus, ropivacaine may have significant therapeutic benefit for patients with cancer who are undergoing cancer surgery by inhibiting CD62E expression and attenuating the adhesion of tumour cell to endothelial cells.

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