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Original article

Montelukast potentiates the relaxing effect of nifedipine in the porcine myometrium

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Abstract

This study analysed the influence of montelukast (MON), a cysteinyl leukotriene receptor antagonist, and nifedipine, an L-type voltage-gated Ca^{2+} channel blocker, on the contractility of the porcine uterine smooth muscle. Myometrial strips were collected from the sexually immature (n=8), cyclic (12-14 days of the oestrous cycle; n=8) and pregnant (27-28 days of pregnancy; n=8) gilts and stimulated with a) MON or nifedipine at concentrations of 10^{-8}-10^{-4} M and b) increasing concentrations of nifedipine after previous administration of MON at a concentration of 10^{-4} M. The changes in the tension, amplitude and frequency of contractions were determined with the Hugo Sachs Elektronik equipment for measuring isometric contractions. Stimulation of the uterine strips with high concentrations of MON significantly reduced the amplitude of contractions in both the cyclic and pregnant group and the frequency of contractions in the pregnant group. In high concentrations, nifedipine significantly decreased the amplitude and frequency of contractions in all examined groups. Nifedipine administered after MON pre-treatment significantly decreased the tension in the pregnant group and the amplitude and frequency of contractions in all examined groups; this effect was more evident in comparison with nifedipine used alone. The results obtained indicate that the influence of MON and nifedipine on the contractile activity of the porcine uterus is dependent on the physiological status of the animal. Moreover, the blockage of the $CysLT_1$ receptor enhances the relaxing effect of nifedipine.

Keywords: montelukast, nifedipine, uterine contractility, gilts

Introduction

It is commonly accepted that the contractility of the uterus is regulated by complex interactions between numerus factors including prostaglandins (Jana et al. 2010, Markiewicz et al. 2016, Malik 2021), lysophosphatidic acid (Markiewicz et al. 2012), acetylcholine, noradrenaline, oxytocin (Markiewicz et al. 2016, Malik 2021), intracellular and extracellular Ca^{2+} (Malik et al. 2021). Incorrect contractility of the uterus results in implantation failure, spontaneous abortions, premature births and other problems in the reproductive tract (Fanchin and Ayoubi 2009). For contractile abnormalities that threaten the maintenance of pregnancy or impede normal delivery, pharmacological agents with different mechanisms of action are used to offset these abnormalities (Buxton et al. 2023). In addition, new therapeutic options that are characterised by effective action and simultaneous limited side effects are being sought. These studies employ a variety of research techniques, taking advantage of the continuous advancement of knowledge in this area (Maxey and McCain 2021). Among the active used/studied substances that regulate uterine contractile activity are leukotriene receptor antagonists such as montelukast and pranlukast (Corriveau et al. 2014, Corriveau et al. 2016) and a calcium channel blocker such as nifedipine (Godfraind 2017, Hyuga et al. 2021).

Leukotrienes (LTs) are synthesised by 5-lipoxygenase (5-LO) and influence the uterus directly through their receptors and indirectly, e.g. via enhancing the action of prostaglandins (Samuelsson 2000). LTs exert their direct effects by binding to and activating membrane receptors: $CysLT_1$ and $CysLT_2$ for leukotriene C_4 (LTC_4), D_4 (LTD_4) and E_4 (LTE_4) (Capra 2004), and BLT_1 and BLT_2 for leukotriene B_4 (Yokomizo and Shimizu 2023). Receptors for LTs were found in the uteri of humans (Corriveau et al. 2014), rats (Corriveau et al. 2016) cattle (Korzekwa et al. 2016), horses (Guzeloglu et al. 2013) and pigs (Jana et al. 2015). LTC_4 and LTD_4 were shown to increase uterine contractility in guinea pigs (Weichman and Tucker 1982) and pigs (Ledwozyw and Kadziolka 1989, Jana et al. 2015) and to modulate the uterine contractility in pregnant women (Corriveau et al. 2010).

Montelukast, a selective, reversible antagonist of $CysLT_1$ receptor, inhibits the 5-LO pathway through $CysLT_1$ receptors, which are coupled to Gq and phospholipase C (PLC) in order to produce inositol trisphosphate (IP_3) (an internal Ca^{2+} release agonist) and diacylglycerol (DAG) (Lipworth 1999). Leukotriene receptor antagonists also present secondary anti-inflammatory effects by inhibiting 5-LO in inflammatory cells (Tomari et al. 2001, Ramires et al. 2004) and interfering with COX-2 activity, resulting in decreased prostaglandin levels (Kahnt et al. 2013). Moreover, specific antagonists of $CysLT_1$ receptors have also been shown to decrease lipopolysaccharides (LPS)-induced cytokines such as TNF-α and IL-6 (Weichman and Tucker 1982, Maeba et al. 2005).

Our previous study demonstrated that montelukast displayed tocolytic properties in an *in vitro* pig model of uterine tissues and caused a significant decrease in the frequency and amplitude of contractions in early pregnant pigs and in amplitude in cyclic pigs (Markiewicz et al. 2018). In other studies, Corriveau et al. (2014) showed that montelukast produced a significant tocolytic effect by decreasing the frequency and area under the curve in the myometrial strips collected from women undergoing elective cesarean sections. Moreover, the addition of montelukast also resulted in a reduced Ca^{2+} sensitivity and an additive effect was observed in combination with nifedipine. Furthermore, in rats following 48 h LPS and montelukast treatment (intraperitoneal four treatments at 12 h intervals), the tocolytic effectiveness of nifedipine was increased (Corriveau et al. 2016). Montelukast can be prescribed for the treatment of asthma in pregnant women, which indicates that its use is safe during pregnancy (Bakhireva et al. 2007, Koren et al. 2010) and also suggests that this antagonist of $CysLT_1$ receptors may be of pharmacological interest in the regulation of uterine contractility.

Nifedipine, an L-type voltage-gated Ca^{2+} channel (L-VGCC) blocker, is one of the most used tocolytics to treat preterm labour (Yart et al. 2022). It limits Ca^{2+} entry into smooth muscle cells in general and myometrial cells in particular, therefore inhibiting muscular contraction (Kuć et al. 2011, Yart et al. 2022). Nifedipine has been used for the past three decades to suppress contractions in preterm labour, and it is still a popular drug because it is cheap and easy to administer.

Taking into account the above, current knowledge about the effect of montelukast administered alone or in combination with nifedipine on uterine contractile activity is insufficient. Therefore, in the presented study, the effect of montelukast and nifedipine administered alone or in combination on the contractile activity of the porcine myometrium collected from immature, cyclic and pregnant animals was examined. The pig model was used for studies because it is commonly used in research on the reproductive tract. Moreover, conducting research on the myometrial strips collected from animals with different physiological statuses will determine whether it has an impact on the action of montelukast and nifedipine.

Materials and Methods

Animals

Tissue samples were harvested from Large White × Polish Landrace gilts intended for commercial slaughter and meat processing, and the collected tissues were an abattoir by-product. Since animal slaughter, tissue collection and transportation of biological material to the laboratory were carried out in accordance with the Polish Act on the protection of animals used for scientific or educational purposes (Anonymous 2015) as well as the Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes (Anonymous 2010), this study did not require the consent of the relevant ethics committee for animal experiments.

The experimental animals were divided into three groups. In the first group, uteri of sexually immature gilts (n=8) with a body weight of 100 to 105 kg were used. In the second group, the uteri of cyclic gilts on 12–14 days of the oestrous cycle (n=8) with a body weight of 110-120 kg were collected; the phase of the oestrous cycle was confirmed on the basis of the morphology of the ovaries (Leiser et al. 1988). In the third group (n=8), uteri were collected from gilts on 27-28 days of gestation (the end of implantation) with a body weight of 122-131; pregnancy was confirmed by washing the horns of the uterus with 10 mL PBS, pH=7.4) to reveal the presence of embryos. The procedure of selection and insemination of the gilts in this group was described previously (Kamiński et al. 2021). Uteri from all animals were collected immediately after slaughter.

Preparation of the uterine strips for measurement of their contraction

Segments of the uterine horns (about 1.5 cm in length), collected from the middle part of the horns, were transferred to ice, moved to the laboratory and immediately processed for examination of contractility. The contractile activity was examined according to the method described previously (Markiewicz et al. 2012, Jana et al. 2013). Strips of the myometrium measuring 3 × 5 mm were suspended in 5 mL of water bath (Schuler Organ bath type 809; Hugo Sachs Electronic, Germany), containing Krebs-Ringer solution (KRs) which consists of (mmol/L): NaCl – 120.3, KCl – 5.9, $CaCl_2$ – 2.5, $MgCl_2$ – 1.2, $NaHCO_3$ – 15.5, NaH_2PO_4 – 1.2, glucose – 11.5, pH 7.4, continuously saturated with carbogen (95% O_2 and 5% $CO_{2)}$. The smooth muscle contractility was determined with Hugo Sachs Elektronik equipment (Hugo Sachs Elektronik) for measuring isometric contractions.

Schedule of contractile activity examination

The recording was started after prior equilibration for at least 60-90 min. At the beginning of the examination, the strips were incubated with increasing (10^{-6}–10^{-4} M) concentrations of acetylcholine (ACh; Sigma, St. Louis, MO, USA) for 15 minutes for each concentration to determine the viability of tissues and their usefulness for further study. Next, the strips were rinsed twice and stimulated as follows: a) montelukast or nifedipine at concentrations of 10^{-8}-10^{-4} M were administered alone at 15-minute intervals after 15 minutes preincubation with KRs, b) montelukast at a concentration of 10^{-4} M was administered 15 min. before administration of increasing concentrations (10^{-8}-10^{-4} M) of nifedipine. The doses of the substances tested were based on previous studies (Corriveau et al. 2014, Markiewicz et al. 2018). Finally, at the end of treatment with examined substances to determine the viability of tissues, ACh was repeatedly administered in the same doses as before. Only those results for which the differences in response to the stimulation by ACh at the beginning and the end of the treatment were less than 20% were included in the statistical analysis. Between each set of examinations, the tissue chambers were washed three times with 15 mL of the incubation solution at 10 min. intervals.

All experimental data were recorded using data acquisition software for tissue bath studies HSE-ACAD® W (Hugo Sachs Elektronik).

Statistical analysis

Numerical values of the contractile activity [tension – changes in resting tension (the preload force which is set by stretching the muscle) expressed in mN, amplitude – the developed force between baseline and maximum of peak expressed in mN, and frequency – number of observed contraction peaks] of the myometrial strips before the application of the examined substances (pre-treatment period) were calculated for 15 min. and accepted as 100%. The results calculated for 15 min. period after treatments were expressed as a percentage of the tension, and frequency and amplitude of contractions measured in the pre-treatment period. The statistical significance of the differences obtained was assessed by a one-way analysis of variance ANOVA (GraphPad Prism 3.1; GraphPad Software, In., San Diego, CA, USA) followed by Bonferroni's multiple comparison test.

Results

Representative diagrams showing contractile activity myometrial strips collected from sexually imma-

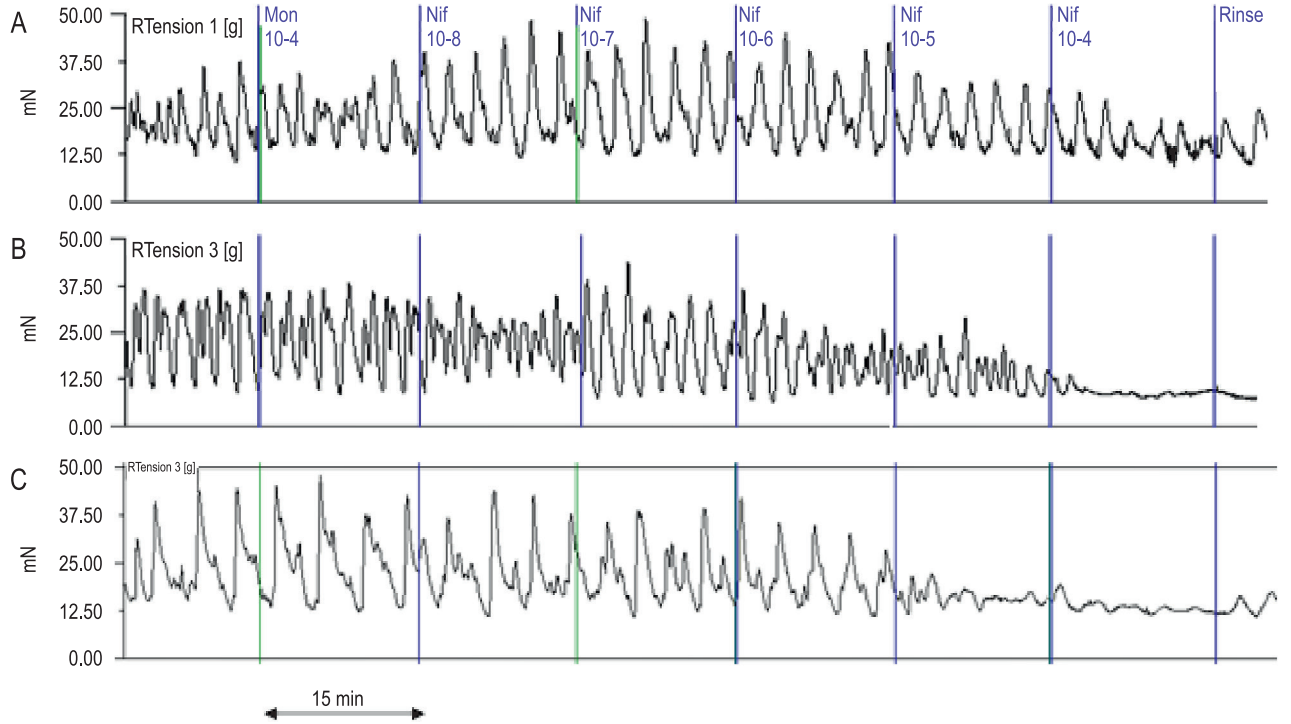

Fig. 1. Representative diagrams schowing the effect of increasing concentrations of nifedipine (Nif) administered after 10^{-4} M montelukast (Mon) pre-treatment in the myometrial strips collected from (A) sexually immature, (B) cyclic (12-14 days of the estrous cycle) and (C) pregnant (27-28 days of pregnancy) gilts.

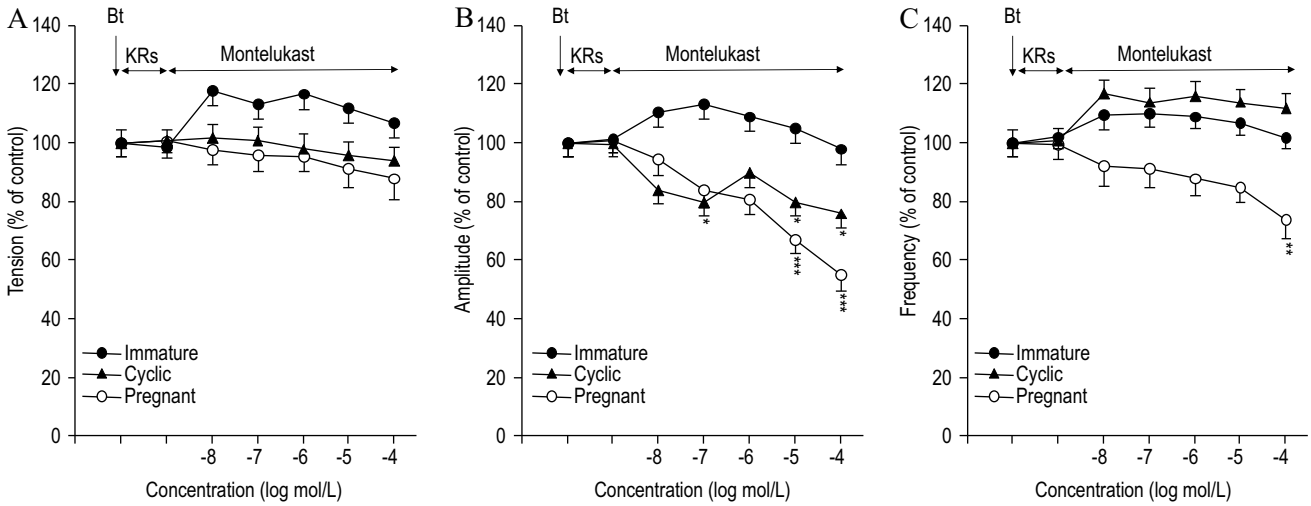

Fig. 2. Effect of increasing (10^{-8}-10^{-4} M) concentrations of montelukast on the tension (A), amplitude (B) and frequency (C) of contractions of the myometrial strips collected from sexually immature (n=8), cyclic (12-14 days of the oestrous cycle; n=8) and pregnant (27-28 days of pregnancy; n=8) gilts. The results calculated for a 15 min period after treatments were expressed as a percentage (mean ± SD) of the tension, and amplitude and frequency of contractions determined for a 15 min period before (Bt) Krebs-Ringer solution (KRs) administration. * $p<0.05$; ** $p<0.01$, *** $p<0.001$ – statistically significant differences compared to the Bt period.

ture, cyclic and pregnant gilts treated with nifedipine pre-treated with montelukast are presented in Fig. 1.

Effect of montelukast

Montelukast did not cause significant changes in the tension in all examined groups (Fig. 2A) as compared to the period before treatment. The tension was significantly lower ($p<0.05$) in the pregnant group as compared to the immature group after montelukast administration at concentrations of 10^{-8} M and 10^{-6}-10^{-5} M (Fig. 2A).

The amplitude of contractions was significantly decreased by montelukast in the cyclic group at concentrations of 10^{-7} M and 10^{-5}-10^{-4} M and in the pregnant group at concentrations of 10^{-5}-10^{-4} M (Fig. 2B) as com-

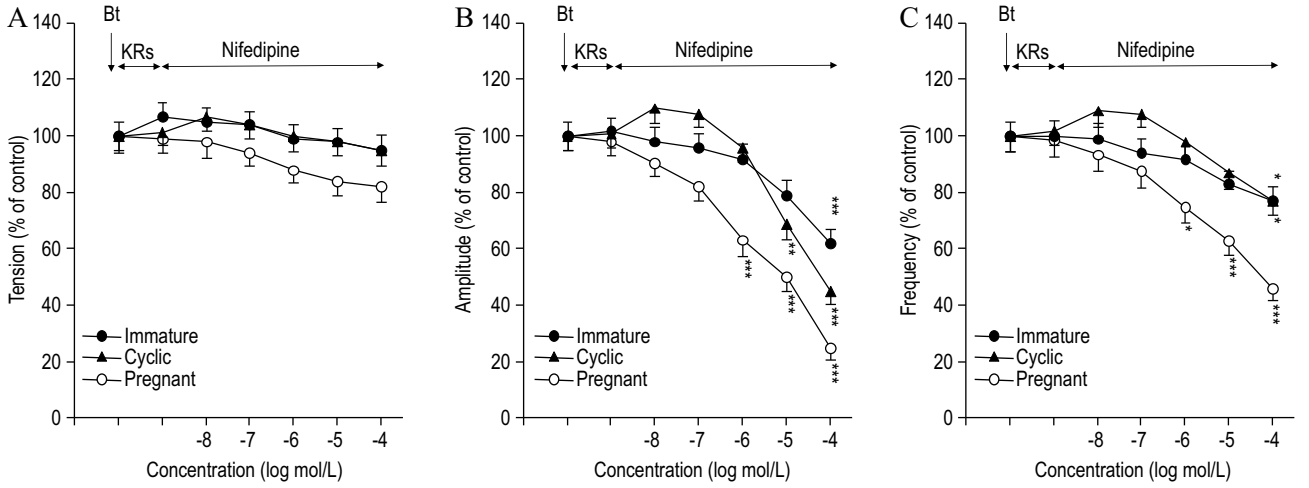

Fig. 3. Effect of increasing (10^{-8}-10^{-4} M) concentrations of nifedipine on the tension (A), amplitude (B) and frequency (C) of contractions of the myometrial strips collected from sexually immature (n=8), cyclic (12-14 days of the oestrous cycle; n=8) and pregnant (27-28 days of pregnancy; n=8) gilts. The results calculated for a 15 min period after treatments were expressed as a percentage (mean ± SD) of the tension, and amplitude and frequency of contractions determined for a 15 min period before (Bt) Krebs-Ringer solution (KRs) administration. * $p<0.05$; ** $p<0.01$, *** $p<0.001$ – statistically significant differences compared to the Bt period.

pared to the period before treatment. The amplitude was significantly lower in the cyclic group at concentrations of 10^{-8} M ($p<0.01$), 10^{-7} M ($p<0.001$), 10^{-5} M ($p<0.01$) and 10^{-4} M ($p<0.05$), and in the pregnant group at concentrations of 10^{-7}-10^{-6} M ($p<0.01$) and 10^{-5}-10^{-4} M ($p<0.001$) as compared to the immature group as well as in the pregnant group at the concentration of 10^{-4} M ($p<0.05$) as compared to the cyclic group.

In the pregnant group, montelukast at the concentration of 10^{-4} M significantly decreased the frequency of contractions as compared to the period before treatment (Fig. 2C). The frequency was significantly lower in the pregnant group at concentrations of 10^{-6}-10^{-5} M ($p<0.05$) and 10^{-4} M ($p<0.01$) as compared to the immature group and at concentrations of 10^{-8}-10^{-7} M ($p<0.05$), 10^{-6} M ($p<0.01$), and 10^{5}-10^{-4} M ($p<0.001$) as compared to the cyclic group.

Effect of nifedipine

Nifedipine did not cause significant changes in the tension in all examined groups (Fig. 3A) as compared to the period before treatment. There were also no significant changes after nifedipine administration at the same concentrations between immature, cyclic and pregnant groups.

The amplitude of contractions significantly decreased after nifedipine administration at the concentration of 10^{-4} M in the immature group, at concentrations of 10^{-5}-10^{-4} M in the cyclic group and at concentrations of 10^{-6}-10^{-4} M in the pregnant group (Fig. 3B) as compared to the period before treatment. The amplitude was significantly lower in the pregnant group at concentrations of 10^{-7} M ($p<0.01$), 10^{-6} M ($p<0.001$) and 10^{-4} M ($p<0.05$) as compared to the cyclic group and at concentrations of 10^{-6}-10^{-5} M ($p<0.01$) and 10^{-4} M ($p<0.001$) as compared to the immature group.

The frequency of contractions was significantly decreased by nifedipine at the concentration of 10^{-4} M in the immature and cyclic group and at concentrations of 10^{-6}-10^{-4} M (Fig. 3C) in the pregnant group as compared to the period before treatment. The frequency was significantly lower in the pregnant group at concentrations of 10^{-7}-10^{-5} M ($p<0.05$) and 10^{-4} M ($p<0.001$) as compared to the cyclic group and at concentrations of 10^{-4} M ($p<0.001$) as compared to the immature group.

Effect of nifedipine after montelukast pre-treatment

Nifedipine administered after montelukast caused a significant decrease in the tension at concentrations of 10^{-6}–10^{-4} M in the pregnant group as compared to the period before treatment (Fig. 4A).

The amplitude was significantly decreased by nifedipine at concentrations of 10^{-8}-10^{-4} M in the pregnant group and at concentrations of 10^{-5}-10^{-4} M in the immature and cyclic group as compared to the period before treatment (Fig. 4B). The amplitude was significantly lower ($p<0.001$) in the pregnant group at concentrations of 10^{-8}-10^{-4} M as compared to the immature group and at concentrations 10^{-8}-10^{-5} M as compared to the cyclic group.

The frequency of contractions significantly decreased after nifedipine administration at concentrations of 10^{-8}-10^{-4} M in the pregnant group and at concentrations of 10^{-5}-10^{-4} M in the immature and cyclic group as compared to the period before treatment (Fig. 4C). The frequency was significantly lower in the pregnant group at concentrations of 10^{-8}-10^{-4} M as compared

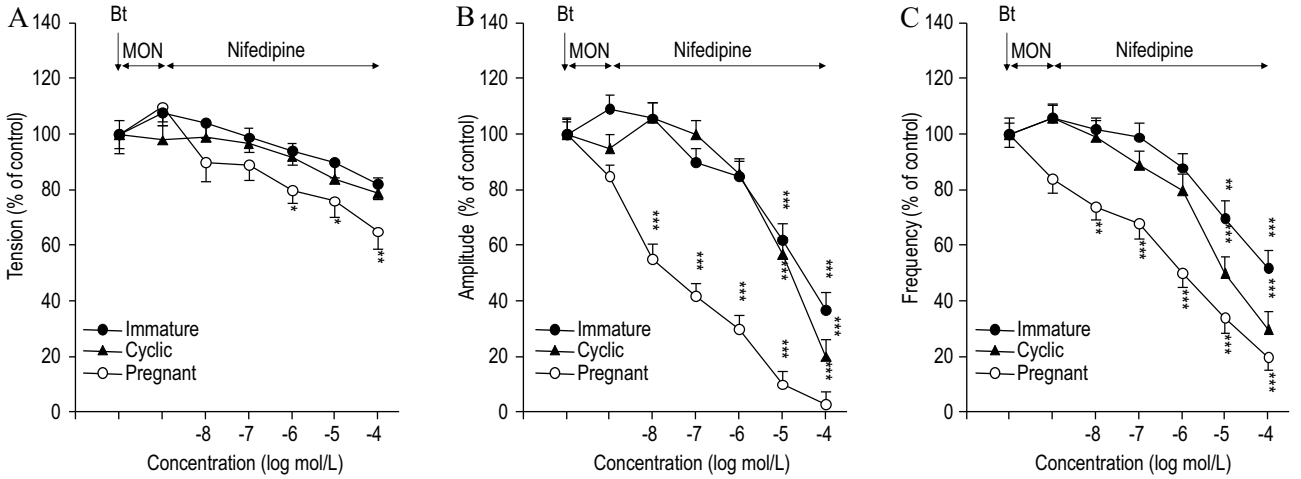

Fig. 4. Effect of increasing concentrations of nifedipine administered after 10^{-4} M montelukast (MON) pre-treatment on the tension (A), amplitude (B) and frequency (C) of contractions of the myometrial strips collected from sexually immature (n=8), cyclic (12-14 days of the oestrous cycle; n=8) and pregnant (27-28 days of pregnancy; n=8) gilts. The results calculated for a 15 min period after treatments were expressed as a percentage (mean ± SD) of the tension, and amplitude and frequency of contractions determined for a 15 min period before (Bt) MON administration. * p<0.05; ** p<0.01, *** p<0.001 – statistically significant differences compared to the Bt period.

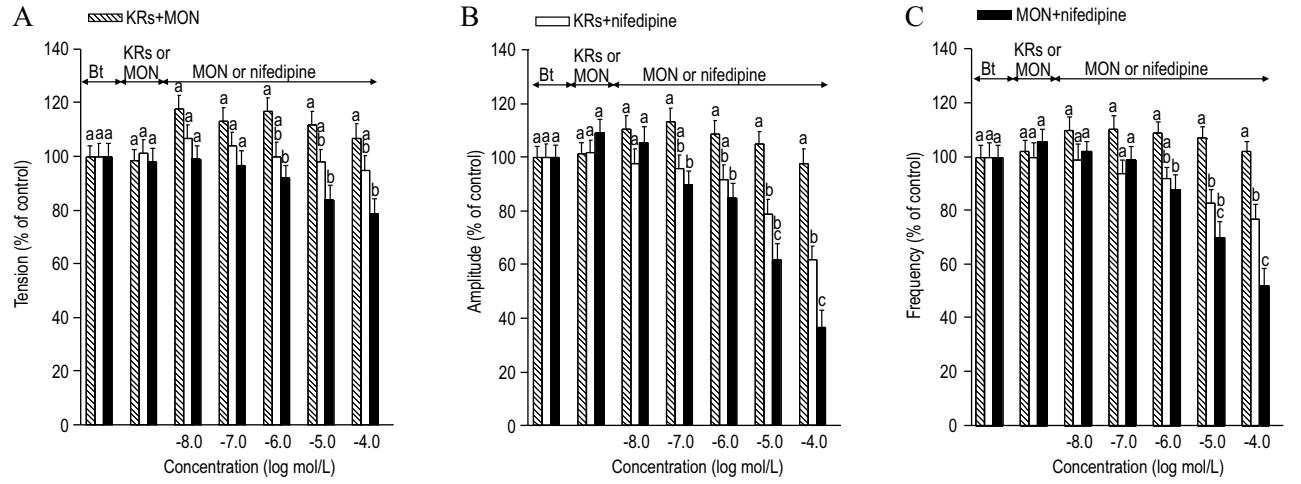

Fig. 5. Effect of increasing concentrations of montelukast (MON) or nifedipine administered after Krebs-Ringer solution (KRs) and nifedipine administered after MON (10^{-4} M) pre-treatment on the tension (A), and amplitude (B) and frequency (C) of contractions of the myometrial strips collected from the sexually immature gilts. The results calculated for a 15 min period after treatments were expressed as a percentage (mean ± SD; n=8) of the tension, and amplitude and frequency of contractions determined for a 15 min period before (Bt) examined substances administration. a,b,c – statistically significant differences (p<0.05-0.001) between KRs and MON as well as MON and nifedipine used at the same concentrations.

to the immature group (p<0.001) and at concentrations of 10^{-8}-10^{-6} M as compared to the cyclic group (p<0.05).

Comparison of the effects of montelukast, nifedipine and nifedipine pre-treated with montelukast

In the immature group there were no significant differences in the tension between montelukast and nifedipine or between nifedipine alone and nifedipine pre-treated with montelukast but the tension was significantly lower (p<0.05-p<0.01) when nifedipine was administered after montelukast at concentrations of 10^{-6}-10^{-4} M as compared to montelukast alone (Fig. 5A). Nifedipine alone caused a significantly higher (p<0.01-p<0.001) decrease in the amplitude at concentrations of 10^{-5}-10^{-4} M as compared to montelukast alone; nifedipine administered after montelukast caused a significantly higher (p<0.05-p<0.001) decrease in amplitude at concentrations of 10^{-7}-10^{-4} M as compared to montelukast alone and at the concentration of 10^{-4} M as compared to nifedipine alone (Fig. 5B). The frequency of contractions was significantly lower (p<0.05-p<0.01) after administration of nifedipine alone at concentrations of 10^{-5}-10^{-4} M as compared to montelukast alone; nifedipine administered after montelukast caused a significantly higher (p<0.05-p<0.001) decrease in amplitude at concentrations of 10^{-6}-10^{-4} M as compared to montelukast alone

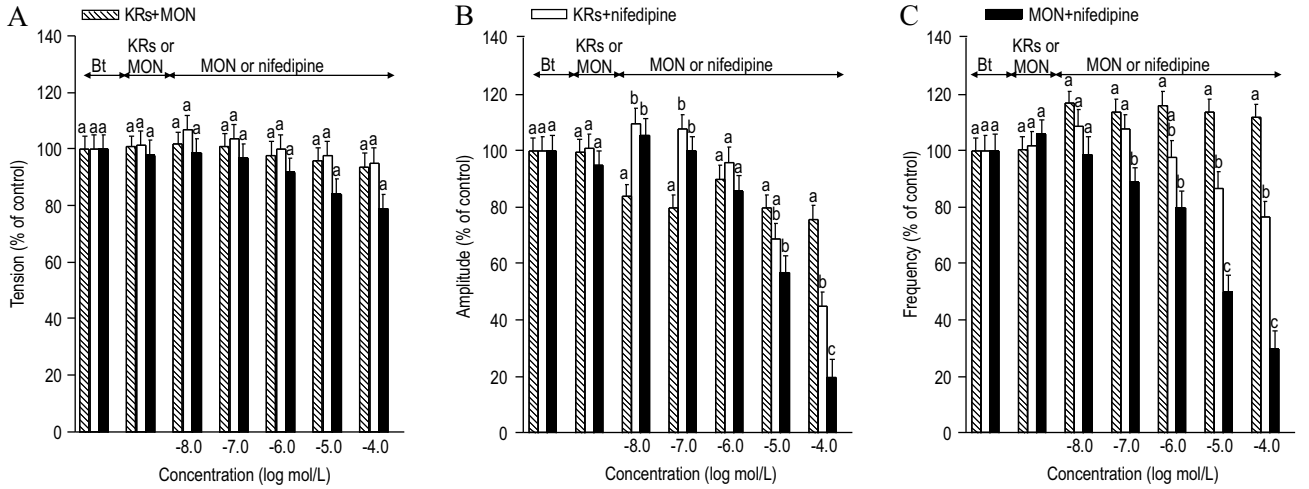

Fig. 6. Effect of increasing concentrations of montelukast (MON) or nifedipine administered after Krebs-Ringer solution (KRs) and nifedipine administered after MON (10^{-4} M) pre-treatment on the tension (A), and amplitude (B) and frequency (C) of contractions of the myometrial strips collected from the cyclic (12-14 days of the oestrous cycle) gilts. The results calculated for a 15 min period after treatments were expressed as a percentage (mean ± SD; n=8) of the tension, and amplitude and frequency of contractions determined for a 15 min period before (Bt) examined substances administration. a,b,c – statistically significant ($p<0.05$-0.001) differences between KRs and MON as well as MON and nifedipine used at the same concentrations.

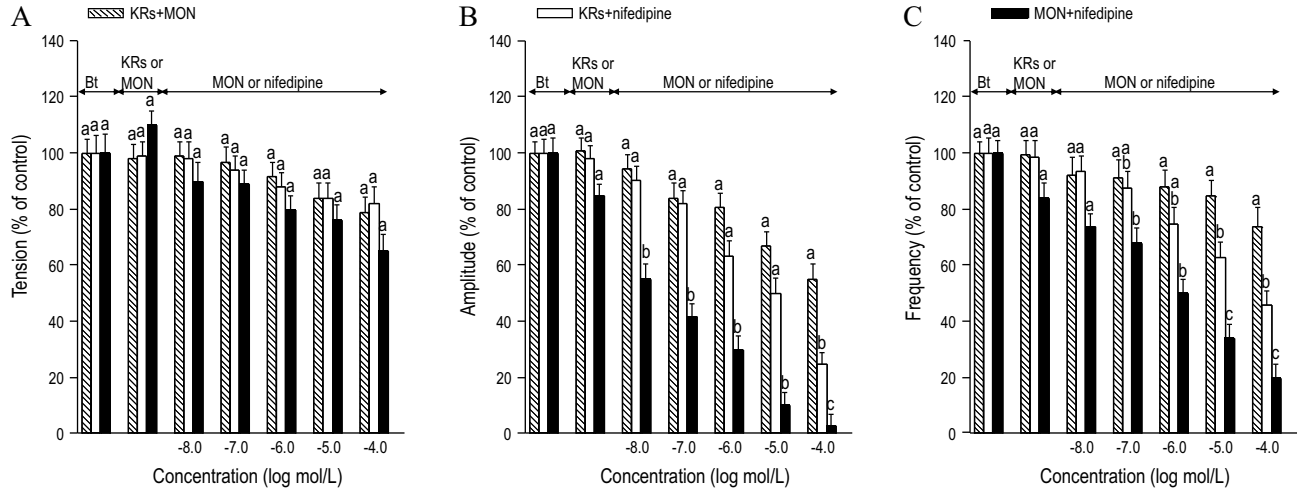

Fig. 7. Effect of increasing concentrations of montelukast (MON) or nifedipine administered after Krebs-Ringer solution (KRs) and nifedipine administered after MON (10-4 M) pre-treatment on the tension (A), and amplitude (B) and frequency (C) of contractions of the myometrial strips collected from the pregnant (27-28 days of pregnancy) gilts. The results calculated for a 15 min period after treatments were expressed as a percentage (mean ± SD; n=8) of the tension, and amplitude and frequency of contractions determined for a 15 min period before (Bt) examined substances administration. a,b,c – statistically significant ($p<0.05$-0.001) differences between KRs and MON as well as MON and nifedipine used at the same concentrations.

and at the concentration of 10^{-4} M as compared to nifedipine alone (Fig. 5C).

In the cyclic group, there were no significant differences in the tension between different treatments (Fig. 6A). The amplitude was significantly lower ($p<0.05$-$p<0.01$) after montelukast administration at concentrations of 10^{-8}-10^{-7} M as compared to both nifedipine alone and nifedipine administered after montelukast but at the concentration of 10^{-4} M the amplitude was significantly ($p<0.05$-$p<0.001$) lower after nifedipine pre-treated with montelukast as compared to nifedipine or montelukast administered alone as well as after nifedipine administration alone as compared to montelukast alone (Fig. 6B). The frequency of contractions was significantly lower ($p<0.05$-$p<0.001$) after nifedipine pre-treated with montelukast at concentrations of 10^{-7}-10^{-4} M as compared to montelukast alone and at concentrations 10^{-5}-10^{-4} M as compared to nifedipine alone as well as after nifedipine administration at concentrations 10^{-5}-10^{-4} M as compared to montelukast alone (Fig. 6C).

In the pregnant group, there were no significant differences in the tension between different treatments (Fig. 7A). Nifedipine administered after montelukast caused a significantly higher ($p<0.001$) decrease in the amplitude at concentrations of 10^{-8}-10^{-4} M as compared

to montelukast alone and nifedipine alone; the amplitude was significantly lower ($p<0.001$) after nifedipine administration alone at the concentration of 10^{-4} M as compared to montelukast alone (Fig. 7B). The frequency of contractions were significantly lower ($p<0.05$-$p<0.001$) after administration of nifedipine pre-treated with montelukast at concentrations of 10^{-7}-10^{-4} M as compared to montelukast alone and at concentrations of 10^{-5}-10^{-4} M as compared to nifedipine alone; nifedipine administered alone at concentrations of 10^{-5}-10^{-4} M caused a significantly higher ($p<0.05$-$p<0.01$) decrease in the frequency of contractions as compared to montelukast alone (Fig. 7C).

Discussion

Nifedipine has been used for many years to suppress contractions in preterm labour because its tocolytic effect is quite well documented. In turn, knowledge about the effect of montelukast used alone or in combination with nifedipine on the myometrium is relatively scarce and is limited to the few available publications. The results of the current study indicate that montelukast and nifedipine administered alone, even in high concentrations, did not change the tension in all examined groups compared to the period before the treatment. However, a marked increase in the tension was observed after montelukast administration in the immature group as compared to the pregnant group. Explaining this phenomenon is difficult due to scarce data on the mechanisms responsible for regulating uterine contractile activity (including data on the representation of leukotriene receptors) in immature pigs. However, it seems probable that after administration of montelukast in the immature group, contractile factors (produced in the arachidonic acid pathway) predominate. In turn, in pregnant animals, the mechanisms responsible for preventing excessive uterine contractions (which could threaten the maintenance of pregnancy) predominate.

Montelukast, despite a lower potency than nifedipine, induced a significant decrease in the amplitude and frequency of contractions in pregnant pigs. Moreover, the apparent additive effect of the association between nifedipine and montelukast is noteworthy. In the case of the tension in the pregnant group and amplitude and frequency of contractions in all studied groups (immature, cyclic and pregnant group), nifedipine action was significantly intensified by montelukast pre-treatment. The obtained data indicate that after the combined administration of montelukast and nifedipine, the tocolytic effect was most evident in the group of gilts in 27-28 days of gestation as compared to the immature and cyclic group, and was more prominent than in earlier studies (Markiewicz at al. 2018) in a group of pigs on 12-14 days of pregnancy. These findings suggest that the porcine myometrium after 27-28 days of gestation is more sensitive to LTs than in the early stages of pregnancy and the luteal phase of the oestrous cycle and immature gilts. The current results confirm the previous observations of Ledwozyw and Kadziolka (1989), who found that pregnant uteri were more susceptible to the action of LTs than non-pregnant uteri, and the response increased parallelly with the advancement of pregnancy. This suggests that persistently high progesterone levels during pregnancy may potentiate the effects of nifedipine and montelukast. This suggestion is supported by porcine myometrial cell studies that have shown that the effects of oxytocin on prostaglandin secretion and Ca^{2+} accumulation are delayed by progesterone during luteolysis and inhibited during pregnancy (Franczak et al. 2006). The current results are also consistent with data obtained by Corriveau et al. (2014) in which the additive tocolytic effect of montelukast in combination with nifedipine was observed in the human myometrial strips collected from women undergoing elective caesarean sections, and uterine tissue collected from rats treated with lipopolysaccharides and montelukast and stimulated *in vitro* with nifedipine (Corriveau et al. 2016).

From the point of view of the mechanism of action, montelukast is known to inhibit the 5-LO pathway through $CysLT_1$ receptors, which are coupled to Gq and PLC in order to produce IP_3 and DAG (Lipworth 1999), whereas nifedipine acts strictly through the blockade of the L-type Ca^{2+}channels (Yart et al. 2022). Thus, the combined use of these compounds with two different modes of action may result in an additive effect. Such an additive effect between montelukast and nifedipine could potentially constitute a complementary treatment. Corriveau et al. (2014) demonstrated that montelukast modulated basal uterine activity and abolished the effects of LTD_4 on the amplitude of phasic contractions. Furthermore, they showed that LTD_4 enhanced either Ca^{2+} entry into myometrial smooth muscle cells (through L-type Ca^{2+} channels), Ca^{2+} release from internal calcium stores, or Ca^{2+}-sensitivity of the contractile machinery via the phosphorylation of regulatory proteins. LTD_4 treatments are known to induce a partial inflammatory condition and enhance contractile responses in various smooth muscle tissues (Ezra et al. 1983, Morin and Rousseau 2007). Moreover, LTD_4 is the metabolite with the highest intrinsic activity toward the $CysLT_1$ receptor (Singh et al. 2006). All of the above indicates that regulation of the $CysLT_1$ receptor activity may be a potential way to modulate uterine contractility.

In summary, the current results demonstrate that blockage of $CysLTR_1$ receptors potentiates the relaxing effect of nifedipine, especially in the uterus of pregnant pigs, which suggests that the influence of these drugs on the contractile activity of the porcine uterus is dependent on the physiological status of the animal. It also suggests that montelukast, in combination with nifedipine, could represent a therapeutic approach to reducing abnormal/excessive contractility of the uterus.

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