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Short communication

Influence of leukotriene receptor antagonist on contractile activity of the porcine uterine smooth muscle in the luteal phase and in early pregnancy

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Abstract

This study analysed the influence of montelukast (MON; 10^{-8} - 10^{-4} M), a cysteinyl leukotriene receptor 1 (CysLTR1) antagonist, on the contractility of the porcine uterine smooth muscle in the luteal phase of the oesterous cycle (n=8) and in early pregnancy (n=8). Stimulation of uterine strips in the luteal phase with MON has been shown to significantly reduce the amplitude of contractions, but not to affect the tension or frequency of contractions. A statistically significant tension increase and decrease in the frequency and amplitude of contractions was observed in pigs in early pregnancy. This suggests that MON has a different effect on the parameters under study in cyclic and pregnant pigs.

Key words: montelukast, cyclic pigs, early pregnancy, uterine contractility

Introduction

Leukotriene receptors were found in the endometrium and myometrium in humans (Corriveau et al. 2014), cattle (Korzekwa et al. 2016), horses (Guzeloglu et al. 2013) and pigs (Jana et al. 2015). Leukotrienes (LTs) C_4 and D_4 were shown to increase the contractility of uterine muscles in guinea pigs (Weichman and Tucker 1982) and in pigs (Ledwozyw and Kadziolka 1989) and to modulate the uterine contractility in pregnant women (Corriveau et al. 2010). In physiological conditions, LTs regulate the function of ovarian follicles and corpus luteum (Blair et al. 1997) and affect the process of embryo implantation (Pakrasi et al. 1985). The time between the 12th and the 14th day of pregnancy in pigs is known to be of key importance for successful implantation, which is associated with the uterus motility and is particularly important for embryo migration.

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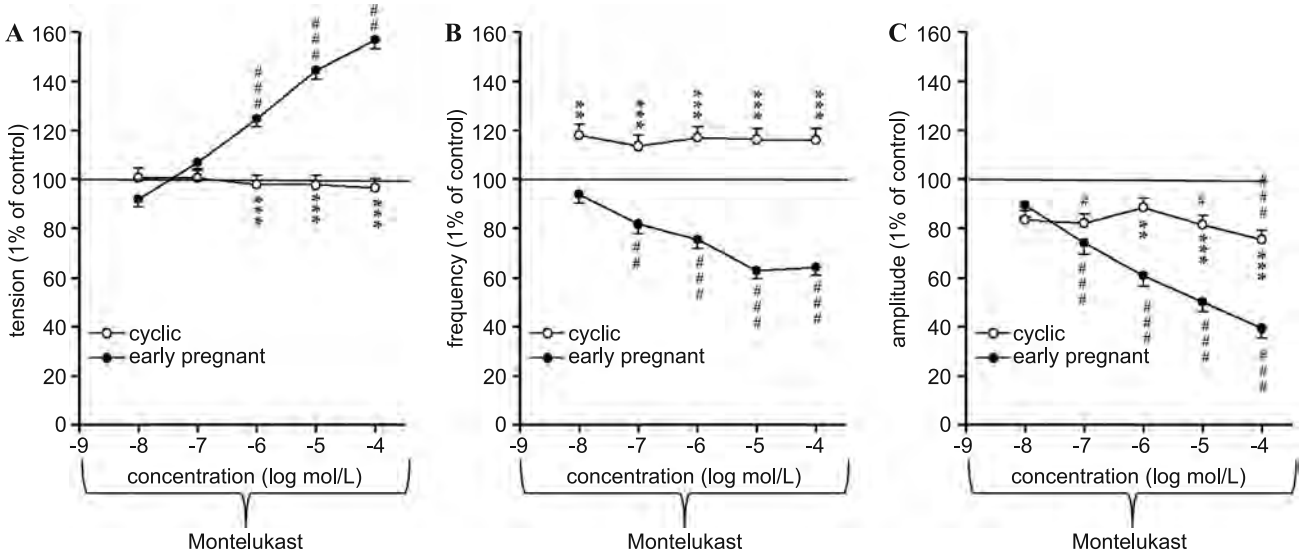

Fig 1. Effect of montelukast, cysteinyl leukotriene receptor 1 (CysLT1) antagonist, on tone (A), frequency (B) and amplitude (C) of contractions of myometrium in pigs on days 12-14 of oesterous cycle (-○- - ; n=8) and on days 12-14 of pregnancy (-●- - ; n=8). The results calculated for a 10 min period after treatments were expressed as a percentage (mean ± SD) of the tension, amplitude and frequency determined for a 10 min period before antagonist administration.
*p<0.05, ** p<0.01, *** p<0.001 – statistically significant differences between the groups of cyclic and pregnant animals following administration of montelukast at the same concentrations,
# p<0.05, ## p<0.01, ### p<0.001 – statistically significant differences compared to the 10-minute period before administration of montelukast.

Improper deployment of embryos in the pig uterus results in their excessively close proximity, which has its consequence in competing for nutrients (Chen and Dziuk 1993, Pere et al. 1997, Rehfeld et al. 2006) and disturbs the development of embryos. However, there are currently no data on the role of LTs receptors in the process. Therefore, the aim of this study was to determine the effect of MON, a CysLTR1 antagonist, on the contractility of smooth muscles in the pig uterus on days 12-14 of the cycle and on days 12-14 of pregnancy.

Materials and Methods

The study was conducted on sexually immature gilts (n=16), with an average body weight of 110-120 kg, in which oestrus was induced by an intramuscular injection of 750 I.U. eCG (Folligon, Intervet, Poland) and subsequently, after 72 hours, of 500 I.U. hCG (Chorulon, Intervet). An intramuscular injection of dinoprost (Dinolytic, Pfizer Trading, Poland), was performed after 13-16 days and eCG was administered again after another 24 hours and hCG – after another 72 hours. Eight gilts were inseminated twice at a 12-hour interval, 24 hours after the hCG administration. The animals were culled on day 12-14 of the cycle (n=8) or pregnancy (n=8; pregnancy was confirmed by washing horns of the uterus with 10 ml PBS, pH=7.4) to wash out the embryos. The uteri were placed on ice and strips of smooth muscles (3 x 5 mm) were collected from the mid-body. Strips of the myometrium (MYO) were suspended in 5 ml of water bath (Schuler Organ bath type 809; Hugo Sachs Electronic, Germany), containing Krebs-Ringer solution which consists of (mmol/L): NaCl, 120.3; KCl, 5.9; $CaCl_2$, 2.5; $MgCl_2$, 1.2; $NaHCO_3$, 15.5; glucose, 11.5; pH 7.4, continuously saturated with carbogen (95% O_2 , 5% CO_2). After 60-90 min. of pre-incubation, the strips under study were stimulated with montelukast (MON; Sigma-Aldrich), at a concentration of 10^{-8}-10^{-4} M, at 15-minute intervals. The smooth muscle contractility was determined with a Hugo Sachs Elektronik equipment for measuring isometric contractions. The experiment was approved by the Local Ethics Commission of the University of Warmia and Mazury in Olsztyn, Poland.

Results and Discussion

Corriveau et al. 2010 demonstrated that inhibition of 5-lipoxygenase (5-LOX) and cyclooxygenase (COX) largely reduced contractions *in vitro* in pregnant human myometrium. Moreover, specific antagonists of CysLTR1, e.g. pranlukast and MON, have also been shown to decrease LPS-induced cytokines such as TNF-α and IL-6 (Maeba et al. 2005). Also, Jana et al. (2015) showed that LTC_4 administered to pigs with an

inflammation of the uterus induced with *E. coli* reduced both the intensity and frequency of contractions, whereas D_4 increased the intensity of contractions and decreased the frequency, which suggests that C_4 is responsible for reduced contractility of the inflamed uterus. Above data indicate that leukotrienes can trigger inflammatory processes (Caldern 2006) and contractile responses (Bryman et al. 1985). The results of the current study indicate that MON causes a significant decrease in amplitude at concentrations of 10^{-7} M and 10^{-5} M ($p<0.05$) and 10^{-4} M ($p<0.001$), but it does not affect the tension or contractions frequency of smooth muscle in the uterus of cyclic pigs (Fig. 1). The tension increase was observed after administration of MON at 10^{-6} - 10^{-4} M ($p<0.001$) at early stages of pregnancy (Fig. 1A), a decrease in frequency at 10^{-7} M ($p<0.01$) and 10^{-6} - 10^{-4} M ($p<0.001$) (Fig. 1B) and of the amplitude at 10^{-7} - 10^{-4} M ($p<0.001$) (Fig. 1C) compared to the period before MON was administered. Moreover, a greater increase was observed after the administration of MON at 10^{-6} - 10^{-4} M ($p<0.001$) at early stages of pregnancy (Fig. 1A), a greater decrease in frequency at 10^{-7} M ($p<0.01$) and 10^{-6} - 10^{-4} M ($p<0.001$) (Fig. 1B) and of the amplitude at 10^{-6} M ($p<0.01$); 10^{-5} - 10^{-4} M ($p<0.001$) (Fig. 1) compared to cyclic pigs. Herein, we demonstrated that MON modulated uterine activity what strongly indicates that CysLTR1 receptors are involved in the regulation of smooth muscle contractility. However, further investigations are needed for the determination whether MON-induced changes are dependent mainly on blocking of LTC_4 or LTD_4 receptors or both of them. In summary, this is the first study showing the effect of MON of the porcine uterine smooth muscle in the luteal phase and in early pregnancy. These findings suggest that myometrium in pigs at early stages of pregnancy is more sensitive to LTs than in the luteal phase of the oestrous cycle, which can affect the process of embryo implantation. Our results confirmed the previous observations made by (Ledwozyw and Kadziołka 1989) where pregnant uteri were more susceptible to leukotriene action than non-pregnant uteri, and response increased parallelly with advancement of pregnancy.

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