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Tocolytic Effect of the Cyclooxygenase-2 Inhibitor, Meloxicam: Studies on Uterine Contractions in the Rat

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Abstract

The objective of this study was to assess the tocolytic activity of meloxicam, a novel cyclooxygenase-2 inhibitor, in a comparative study with the non-steroidal anti-inflammatory drug, indomethacin.

The in-vitro tocolytic effect of meloxicam and indomethacin (10^{-9}–10^{-5} M) was evaluated on spontaneously-contracting uterine strips from non-pregnant rats and from rats at various stages of pregnancy. The rhythmic motility of the strips was reproducibly maintained over several hours. The effect of drugs was evaluated from the extent of inhibition of the frequency and amplitude of contractions. Both indomethacin and meloxicam induced dose-dependent inhibitory effects, with meloxicam being slightly more potent in all groups studied, particularly in early pregnancy.

These results suggest that meloxicam, which has fewer side-effects than cyclooxygenase-1 inhibitors, could be a potentially useful tocolytic agent in the treatment of premature labour.

Prostaglandins are important in the mediation of several key processes during pregnancy and parturition (Kelley 1994). The biochemical mechanisms of the initiation of labour have been studied in several animal models and it is now generally accepted that an increase in prostaglandin synthesis within the uterus is an essential component of the parturition process (Bennet & Elder 1988). Prostaglandin synthesis is controlled by two rate-limiting enzymes, phospholipase A_2 and cyclooxygenase (COX), also referred to as PGH_2 synthetase (Romero & Behrman 1991). Two distinct isoforms of COX have been identified and are encoded by two different genes (Wen et al 1993). The anti-inflammatory effects of non-steroidal anti-inflammatory drugs are a result of inhibition of the inducible isoform COX-2 whereas many of the side-effects of these drugs result from inhibition of COX-1 (Copeland et al 1994).

Pregnancy can be prolonged by inhibition of prostaglandin synthesis in man (Topozoda et al 1984). However, the pharmacological usefulness of this approach has so far been limited by side-effects on foetal development, including premature closure of the ductus arteriosus (Moise 1983; Romero & Behrman 1991). Antenatal use of indomethacin also carries the risk of neonatal cerebral haemorrhage and of necrotizing enterocolitis (Enkin et al 1995). Each of these side-effects is presumably a result of the inhibition of the constitutively produced prostaglandins which are the products of COX-1. It might, therefore, be predicted that the therapeutic use of COX inhibitors such as meloxicam, which are specific for COX-2, should effectively suppress the contractions of pre-term labour without causing the foetal side-effects of indomethacin (Bennet & Slater 1996). In support of this hypothesis it has been shown that expression of COX-1 is greater than that of COX-2 in foetal heart, lung, kidney and brain, and that COX-2 expression in these tissues is some 1000-fold less than in the foetal membranes at term (Bennet & Slater 1996). If the activation of COX-2 enzyme expression and activity is an essential step in triggering parturition, specific inhibitors of COX-2 should be promising tools for the treatment of premature labour.

In this study our objective was to investigate the uterine inhibitory effect of meloxicam, a COX-2 inhibitor, in comparison with a typical non-steroidal anti-inflammatory drug (NSAID), indomethacin. The selectivity of meloxicam in inhibiting COX-2, but not COX-1, might provide a useful drug for the treatment

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of pre-term labour with less serious side-effects, especially for the foetus.

Materials and Methods

Experiments were performed on mature female Sprague–Dawley rats including non-pregnant, early pregnant (6–7 days), mid-pregnant (13–14 days) and late pregnant (20–21 days) animals. The rats were killed by decapitation under light ether anaesthesia and 15–20-mm strips were cut from the uterus and suspended in Krebs–Henseleit solution at pH 7·4 in 25-mL organ baths (95% O_2–5% CO_2, 37 °C). Isometric tension was recorded on a Lectromed UFI-dynamometer and recorder system. The preparations were left for 30 min, the Krebs solution being changed at 15-min intervals. Thereafter a pre-tension of 1·5 g was applied and the preparations were left for a stabilization period of approximately 45 min. After a stable pattern of rhythmic contractions had been established, a cumulative dose–response curve was constructed for meloxicam or indomethacin (10^{-9}–10^{-5} M). The preparations were incubated with each concentration of the inhibitors for 30 min, the procedure employed being similar to the technique previously used for specimens from man (Thulesius et al 1987). Changes in frequency (contractions min^{-1}) were assessed by comparing the total number of contractions by the end of the 30-min period after administration of each concentration of the drugs. Changes in the amplitude (mm) of the contractions after incubation with the drugs were also recorded. We also measured the time until complete stoppage of rhythmic contractions after administration of the last dose of meloxicam or indomethacin. Dose–response curves were analysed by use of Graph-Pad Prism software. Results are presented as means ± s.e. (n = 5–7). Standard statistical evaluation of the data was performed with the Instat program; $P < 0.05$ was taken as indicative of significance.

Drugs and chemicals

The composition of the Krebs–Henseleit solution was (mM): NaCl (118·3), KCl (4·7), $CaCl_2$ (2·5), $MgSO_4$ (1·2), $NaHCO_3$ (25), KH_2PO_4 (1·2) and

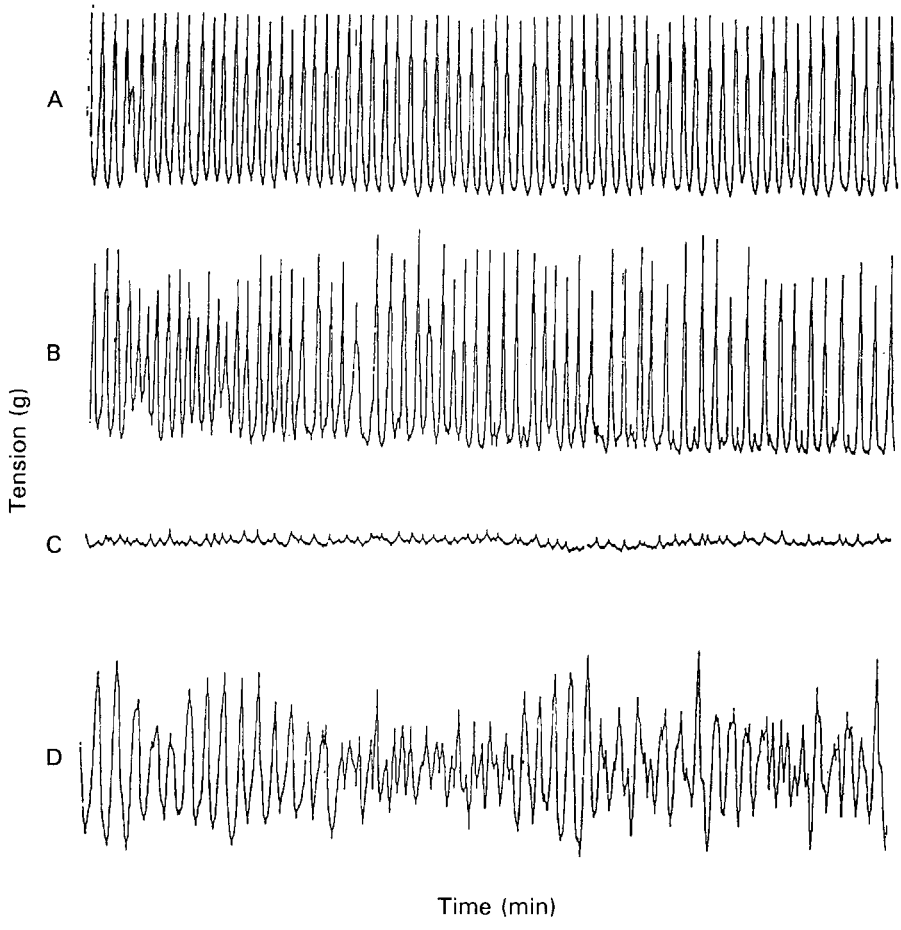

Figure 1. Original traces of the spontaneous contractile activity of the uterus at the different stages of pregnancy. A. Non-pregnant; B. early pregnancy; C. mid-pregnancy; D. late pregnancy. The horizontal scale represents time (min) and vertical scale represents tension (g). For A, C and D the horizontal unit is 1 min and vertical unit is 0·5 g; for B they are 2 min and 1 g, respectively.

Table 1. Frequency and amplitude of uterine contractions recorded before administration of the drugs at different stages of pregnancy.

	Frequency (contractions min^{-1})	Amplitude (mm)
Non-pregnant	1·6±0·1	27±3
Early pregnancy	4·7±0·9	116±15
Late pregnancy	1·9±0·7	55±25

Values are mean±s.e., n=7.

glucose (11·2). Indomethacin was obtained from Research Biochemicals International (RBI; Natick, MA), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from Sigma (St Louis, MO). Meloxicam was a gift from Dr Karl Thomae (Germany). Indomethacin was dissolved in methanol, meloxicam in *N,N*-dimethylformamide. The final concentration of the solvents in the organ baths in all the experiments did not exceed 0·1%, which did not have any effect on tissue contractility (tested in preliminary experiments).

Results

In most preparations rhythmic contractile activity was observed to occur spontaneously, shortly after mounting the uterine strips. The spontaneous contractions were reproducible over a period of 4–5 h. The pattern of this rhythmic contractility is shown in Figure 1. The characteristics of the rhythmic contractile activity at the various stages studied are shown in Table 1. The mid-pregnancy specimens were almost inactive; only very low amplitude spontaneous irregular activity was observed unless the strips were activated by administration of $PGF_{2\alpha}$ (10^{-6} M).

Figures 2 and 3 show dose–response curves of the effect of increasing concentrations of meloxicam and indomethacin against the frequency and amplitude of uterine contractions. These diagrams show that both compounds dose-dependently inhibit uterine contractions in non-pregnant rats and also during pregnancy. It is apparent that the frequency of uterine contractions becomes significantly less after incubation with micromolar concentrations of meloxicam than after indomethacin, higher concentrations of which were usually required to produce similar effects (Figure 2). A similar response pattern is evident from Figure 3, which shows the effect of meloxicam and indomethacin on the amplitude of uterine contractions during the different gestation periods. The difference between the two compounds becomes particularly evident in early pregnancy. It is apparent that changes in the amplitude of the uterine contractions in response to the inhibitors

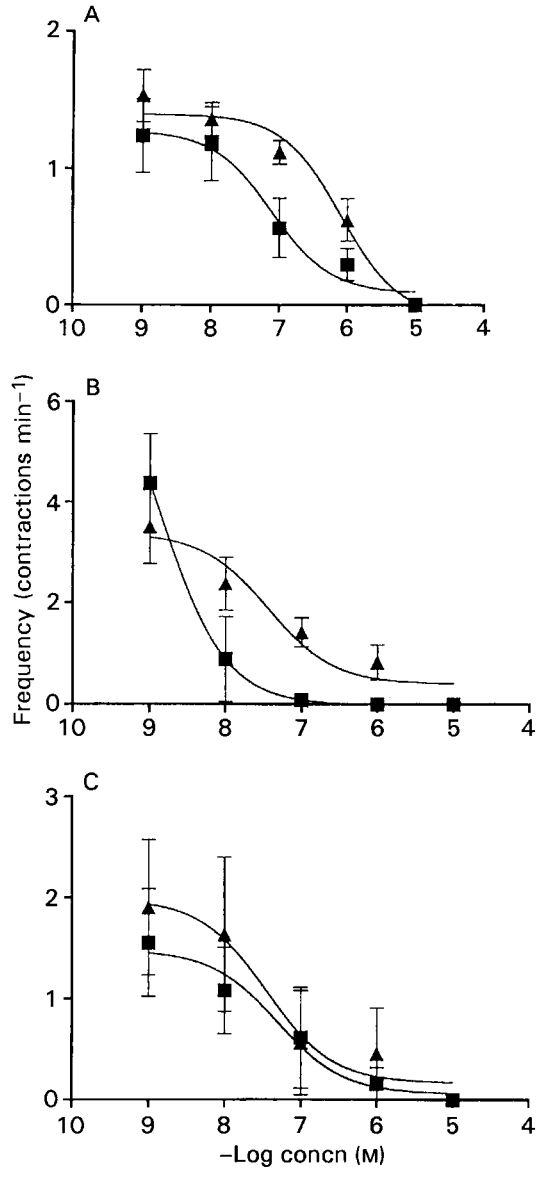

Figure 2. Frequency-response relationship for the effect of meloxicam (■) and indomethacin (▲) (10^{-9}–10^{-5} M) on spontaneous contractions of the uterus of rats (n=5). A. Non-pregnant; B. early pregnancy; C. late pregnancy.

were similar in pattern to the changes in frequency. In general it was found that meloxicam induced inhibition at lower concentrations than indomethacin in all gestation periods.

The potency (− log molar concentration) of meloxicam and indomethacin in terms of changes determined in the frequency of contractions or amplitude was measured from the dose–response curves established for both drugs. As shown in Table 2, the potency of meloxicam, recorded from the frequency dose–response curve, in inhibiting spontaneous uterine contractions was slightly greater than that of indomethacin in the different gestation periods studied and particularly in early pregnancy. A similar result for the potency of

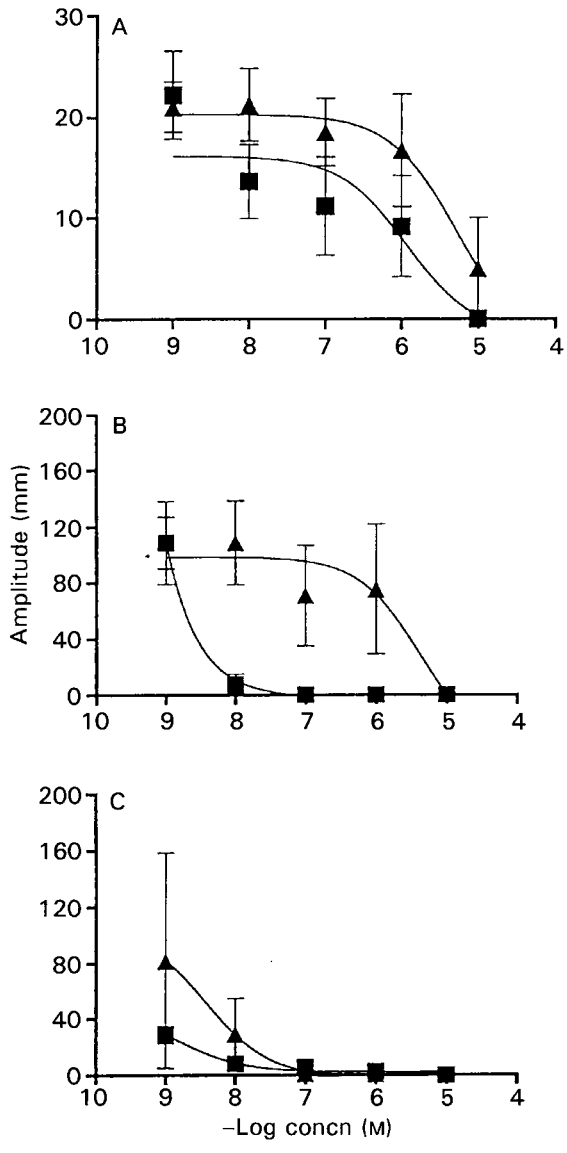

Figure 3. Amplitude–response relationship for the effect of meloxicam (■) and indomethacin (▲) (10^{-9}–10^{-5} M) on spontaneous contractions of the uterus of rats (n = 5). A. Non-pregnant; B. early pregnancy; C. late pregnancy.

Table 2. The potency of meloxicam and indomethacin determined from the frequency and amplitude dose–response curves for both drugs at different stages of pregnancy.

	Meloxicam	Indomethacin
Frequency (contractions min^{-1})		
Non-pregnant	7·1 ± 0·3	6·1 ± 0·2
Early pregnancy	8·9 ± 0·7	7·4 ± 0·4
Late pregnancy	7·3 ± 0·6	7·4 ± 0·7
Amplitude (mm)		
Non-pregnant	5·9 ± 0·6	5·3 ± 1·1
Early pregnancy	9·96 ± 3·96	5·4 ± 1·1
Late pregnancy	8·8 ± 0·6	8·4 ± 1·1

Potency = concentration inducing half the maximum response, EC50 (− log molar concentration) (n = 5–7).

uterine contractile activity was also apparent from the amplitude dose–response curve. The time needed for the drugs to totally abolish uterine activity (stop-time, min) was also calculated. For all the different stages studied the stop-time for meloxicam (154·5, 121·0 and 140·8 min for non-pregnant rats, for early pregnancy and for late pregnancy, respectively) was shorter than for indomethacin (185·8, 144·9 and 145·3 min, respectively).

Discussion

In various species, including rodents (Saksena et al 1976; Rankin et al 1979; Kennedy 1985), prostaglandins affect several biological systems including the uterus. That prostaglandin levels in the circulation rise during labour (Bennet & Slater 1996) is supported by the observation that there is an increase in uterine COX-2 gene expression towards term; this might be important in the mediation of increased uterine $PGF_{2\alpha}$ production and, in turn, luteolysis and parturition (Arslan & Zingg 1996).

The technique we used to study tocolytic activity on rat uterine strips is basically the same as that used in previous experiments on material from man (Thulesius et al 1987). The rhythmic contractions of characteristic frequency and amplitude observed for uterine muscle preparations seemed to reflect the in-vivo situation. Standardization of the procedure made it possible to evaluate drugs with tocolytic activity by measuring changes of contraction frequency and amplitude. Our study shows that both meloxicam and indomethacin dose-dependently block both frequency and amplitude of the contractions and can even induce total stoppage. This means that these drugs significantly interfere with the de-novo synthesis of prostaglandins in the uterine preparations. The potency ratio of meloxicam in comparison with that of indomethacin in its effect on COX-2 is more than 60-times higher than that for COX-1 (Vane & Botting 1996). It has previously been shown that indomethacin is highly effective in preventing the contractions of pre-term labour and more effective in prolongation of pregnancy than the more commonly used β-sympathomimetics (Bennet & Slater 1996). This means that uterine motility is also governed by COX-1 activity, a finding supported by our study.

The use of indomethacin is limited by foetal side-effects such as reduced foetal urine output leading to oligohydramnios (Kirshon et al 1991). There is also increased risk of constriction or closure of the ductus arteriosus in foetuses whose mothers have received high-dose indomethacin therapy (Moise 1983). Each of these side-effects is presumably because of inhibition of constitutively produced prostaglandins, which would therefore be products

of COX-1 (Bennet & Slater 1996). It might therefore be predicted that the therapeutic use of COX inhibitors which are specific for COX-2, should effectively suppress the contractions of pre-term labour without causing the foetal side-effects seen with indomethacin. In support of this hypothesis we have shown that meloxicam is at least as potent as indomethacin in inducing tocolytic activity on uterine contractions of the rat at different stages of gestation; and the stop-time of meloxicam was always shorter than that for indomethacin.

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