Interleukin-8 potentiates the effect of interleukin-1-induced uterine contractions

Selina Khatun, Naohiro Kanayama¹, Hossain Md Belayet, Masumi Yonezawa, Takao Kobayashi and Toshihiko Terao

Department of Obstetrics and Gynaecology, Hamamatsu University School of Medicine, 3600 Handa Cho, Hamamatsu City 431-3192, Shizuoka Prefecture, Japan

¹To whom correspondence should be addressed

The aim of this research was to study the effect of exogenous interleukin (IL)-8, IL-1 and IL-8 + IL-1 on uterine contractions in rabbits. Four equal groups of non-pregnant rabbits (n = 24) were investigated using either placebo or experimental drugs in the form of vaginal suppositories. The suppositories contained human recombinant IL-8 (200 ng), IL-1 (200 ng), IL-8 (200 ng) + IL-1 (200 ng) or vehicle (Witepsol base, 500 µl). Subsequently, the plasma concentration of prostaglandin (PG) E₂ was estimated 3 h after the last dose of treatment. Neutrophil infiltration in the endometrial tissue was studied with anti-rabbit RT2 staining. Suppositories with IL-1 and IL-8 + IL-1 induced contractile responses with increased frequency (P < 0.003, P < 0.0005) and amplitude (P < 0.0001) in vivo, compared with vehicle. IL-1 and IL-8 + IL-1 also caused similar contractile effects with increased frequency (P < 0.01, P < 0.0007) and amplitude (P < 0.0001) in an in-vitro experiment than vehicle. The frequency and amplitude of uterine contractions were more significant with IL-8 + IL-1 than that of IL-1, both in vivo (P < 0.002, P < 0.05) and in vitro (P < 0.005, P < 0.01). IL-8 did not induce any contractions. Prostaglandin concentration was increased ~8-fold with IL-8 + IL-1 (P < 0.0001) and 2.5-fold with IL-1 treatment (P < 0.0001). Neutrophil numbers were significantly increased with IL-8 + IL-1 > IL-8 > IL-1 (P < 0.002, P < 0.0003 and P < 0.008) compared with vehicle. Our data suggest that IL-8 stimulates IL-1-induced uterine contractions through PGE₂ production and could be an important process during labour and delivery.

Key words: interleukin-1/interleukin-8/neutrophil influx/prostaglandin E₂/uterine contraction

Introduction

The maintenance of pregnancy and the initiation of parturition are closely related to timely regulation of biomolecular processes, in the connective tissue of the reproductive tract (Casey and MacDonald, 1988). Biochemical and cellular changes that occur in the endometrial tissues at the time of labour are similar to those that occur with inflammatory processes. Such inflammatory responses include infiltration of white blood cells (Liggins, 1981). Neutrophil activation is the penultimate antecedent event in the initiation of labour. It has been suggested that these cells act as physiological agents responsible for the connective tissue changes which take place during the ripening process (Junqueira et al., 1980; Osmers et al., 1992). The most likely agent to induce neutrophil infiltration into the tissue is interleukin-8 (IL-8). This compound, a member of the chemokine family, is chemotactic for neutrophils (Baggiolini et al., 1989) and causes them to degranulate within tissues. As parturition involves neutrophil migration into the tissues, IL-8 is an important mediator of the tissue rearrangements that accompany these events (Kelly, 1994). Recently several reports have emphasized that the inflammatory cytokine IL-8 is produced from cultured amnion, chorion and decidual cells constitutively and in response to other cytokines (Kelly et al., 1992; Trautman et al., 1992). IL-8 concentration in the amniotic fluid increases gradually in the third trimester of pregnancy (Laham et al., 1993). Evidence has accumulated that this potent agent has many physiological roles in the process of parturition (El Maradny et al., 1994, 1995; Kelly, 1994).

IL-1 is a hormone-like polypeptide that apparently performs many roles in inflammation and immunity. This peptide is synthesized and secreted by chorio-decidual macrophages during parturition (Romero et al., 1989d), and has been implicated as a signal for the onset of labour (Romero et al., 1989a, 1990, 1991; Mitchell et al., 1990). IL-1 has numerous biological effects on various target cells (Dinarello, 1988). IL-1 induces IL-8 production (Uchiyama et al., 1992), activates neutrophils (El Maradny et al., 1995), regulates prostaglandin (PG) biosynthesis (Casey and MacDonald, 1988; Romero et al., 1989b; Mitchell et al., 1990) and increases the production of other cytokines which are capable of stimulating prostaglandin production by intrauterine tissues (Romero et al., 1992c; Mitchell et al., 1991b). PGE₂ is an inflammatory mediator known to be involved in myometrial contraction during labour (Barclay et al., 1993). Additionally, IL-8 and PGE₂ have been found to act synergistically, PGE₂ reducing the concentration of IL-8 required to cause neutrophil invasion of rabbit skin (Colditz, 1990). Previously, we reported that exogenous IL-8 or IL-1 induces cervical dilatation in rabbit (El Maradny et al., 1994, 1995). However, there have been no reports about the effect of IL-8 to IL-1 on uterine muscle contraction. We postulated that the changes in IL-8 concentrations in the endometrial tissue may potentiate the effect of IL-1-induced uterine contraction. The purpose of this study was to determine the effect of exogenous IL-8 on IL-1-induced uterine contraction in rabbits.

560

© European Society of Human Reproduction and Embryology
Materials and methods

The protocol of this research was approved by the Research Committee of Laboratory Animals of Hamamatsu University. The research was carried out on 24 sexually mature Japanese white female non-pregnant rabbits (SLC, Hamamatsu, Japan) weighing 3.0–3.5 kg. Rabbits were caged under controlled temperature and light and were given Purina rabbit chow (Clea Japan Inc., Tokyo, Japan) and water ad libitum. The rabbits were comparable in age and weight. Suppositories were prepared with 500 μl Witepsol-50 bases (WT-50, Adeps solidus: Mitsuba Co., Tokyo, Japan), the products of cocoa butter of plant origin. The suppositories using Witepsol bases were quick to melt and poor in attaching to the tissue. The release of drug from the suppository was measured by a recommended method and we found that drug was released from the suppositories over a short period.

Experimental design

The animals were divided equally into four groups. The first group (IL-8 group), was treated by suppository containing 200 ng recombinant human IL-8 (Oncogen Science Inc., MA, USA) for 3 days. IL-1 (200 ng: Oncogen Science) was applied to each rabbit for 1 day in the second group (IL-1 group). Human recombinant IL-8 and IL-1 cross-react easily in rabbits. Three day treatment with IL-8 is essential to obtain the desired effect and 1 day treatment with IL-1 was found to be sufficient to produce optimal uterine contraction. Rabbits in the third group (IL-8 + IL-1 group) received suppositories containing IL-8, 200 ng for 3 days followed by IL-1, 200 ng. The fourth group (vehicle group) was treated with 500 μl of Witepsol base (Adeps solidus: Mitsuba Co., Tokyo, Japan). This vehicle group was considered the sham control. The uterine contraction in rabbits was also measured without any treatment, and the effect was found to be quite similar to that of the vehicle group.

Plasma collection

Peripheral blood (1 ml) was gently drawn from the middle ear artery into tubes (containing EDTA + 2Na, indomethacin and aprotinin) 3 h after the last dose of suppository treatment. The blood was centrifuged at room temperature for 10 min at 2000 × g. Plasma centrifugates were frozen immediately at −70°C until analysis. Plasma concentrations of PGE 2 were detected by enzyme-linked immunosorbent assay (BIOTRA; Amersham Life Science, London, UK) according to the manufacturer’s instructions. The concentration of PGE 2 was determined from a standard curve derived from PGE 2 standard, which was run with each assay. All determinations of plasma PGE 2 were carried out in duplicate.

In-vivo measurement of isometric contraction force

After blood sampling, in-vivo uterine contractions were measured as described previously (Machino et al., 1997). Briefly, surgery was conducted on a servo-controlled heating table to maintain body temperature at a constant 37°C. Anaesthesia was induced with ketamine hydrochloride (30 mg/kg, Ketalar; Parke Davis, Tokyo, Japan), injected through a marginal ear vein. Two PE-50 catheters were placed in the left jugular vein. One catheter was used for continuous infusion of Ketalar (6–8 mg/kg/h) and another for infusion of saline. The animals were allowed to breathe spontaneously. The uterus was exposed through a lower midline abdominal incision. The force transducer (NEC, Tokyo, Japan) was attached to the anterior surface of the body of the uterus using 3–0 suture material. These sutures were used to anchor the transducer cable to the uterus. The transducer was connected to a polygraph recorder (RMG 5204, Nihon-Kohden, Tokyo, Japan) via a universal amplifier. The transducer was standardized and 30 min were allowed for stabilization. Measurements were made after reaching stable uterine contraction. Contraction responses were measured isometrically for 30 min and recorded on a polygraph. Since the force transducer cannot give the absolute values of contraction the data were expressed as g weight that equals to 0.026 mV. All procedures were performed with strict aseptic conditions.

Sample preparation

The rabbits were killed with an overdose of Ketalar immediately after the experiments. After careful dissection, the uterus was divided into two parts; one half was placed in oxygenated buffer for the measurement of uterine contraction (in vitro) and another half was placed in 10% formalin for histological sections. The uterine muscles were immediately immersed in chilled, oxygenated Krebs–Ringer bicarbonate solution. Uterine muscle fibres were cut into 3×7 mm strips under a stereomicroscope. The myometrial strips were suspended in temperature-controlled organ baths containing 15 ml of Krebs–Ringer bicarbonate-buffered solution of the following composition (mM): NaCl 113, KCl 4.8, CaCl2 2.2, KH2PO4 1.2, MgSO4 1.2, NaHCO3 25, and glucose 5.5. The organ baths were oxygenated with 95% O2–5% CO2 at 37°C.

In-vitro recording of isometric force development

The isometric contraction of the myometrium was measured with a force-displacement transducer (TB-612T, Nihon-Kohden) connected to a MacLab Dose Response (AD Instruments, Tokyo, Japan) computer-based data acquisition system. Silk ligatures were tied to each end of the strips and one end of each strip was fixed to a Plexiglass rod and mounted in the organ baths irrigated with Krebs–Ringer buffered solution. The other end of each strip was connected to the force-transducer. A resting tension of 1 g was applied to the strips, and the isometric tension of the myometrial strips was recorded for 6 h.

Immunohistochemistry and quantification of stained cells

Histological sections were stained immunohistochemically for the surface antigen RT2, found in rabbit leukocytes using anti-rabbit RT2 monoclonal antibody (Cedar lane Laboratories, Hornby, Canada) as described previously (Ponsard et al., 1986). Paraffin embedded tissue sections were deparaffinized in xylene baths and rapidly rehydrated through graded 95% alcohol. Excess liquid was removed, and sections were washed in phosphate buffered saline (PBS, pH 7.2). Endogenous peroxidase was blocked by fixation of the sections in methanol and 3% hydrogen peroxide for 20 min at 23°C. To block the tissue sections bovine serum albumin–phosphate buffered solution (BSA–PBS, 2%) was used. Anti-rabbit RT2 (1:100) monoclonal antibodies were placed on the sections after 20 min, and kept overnight at 4°C. The sections were incubated with the second antibodies (goat anti-rabbit IgG antibody; DAKO, USA) at 1:100 dilution for 2 h at room temperature, after washing 5 times in PBS. Avidin–biotin–peroxidase complex (DAKO) was added at 1:1000 dilution for 2 h at room temperature, after washing 3 times in PBS. Anti-RT2 RT2 (1:100) monoclonal antibodies were placed on the sections after 20 min, and kept overnight at 4°C. The sections were incubated with the second antibodies (goat anti-rabbit IgG antibody; DAKO, USA) at 1:100 dilution for 2 h at room temperature, after washing 5 times in PBS. Histological sections were dehydrated, then examined by light microscopy at ×200 magnification. Negative controls received the same treatment without primary antibody. Neutrophils in the tissue were used as positive control. The number of neutrophils was estimated by counting the number of stained extravascular cells within a line grid (10×10 squares) occupying an area on the section of 0.1–5 mm2 using a ×20 objective and ×10 eyepiece and the mean values were calculated.
Table I. Contractions evoked by suppositories containing interleukin (IL)-8, IL-1, IL-8 + IL-1 and vehicle in rabbit uterus

<table>
<thead>
<tr>
<th>Uterine contraction</th>
<th>In vivo</th>
<th>In vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency/10 min</td>
<td>Amplitude g</td>
</tr>
<tr>
<td>IL-8 (200 ng)</td>
<td>9.3 ± 2.6</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>IL-1 (200 ng)</td>
<td>23.8 ± 4.3*</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>IL-8 (200 ng) + IL-1 (200 ng)</td>
<td>36.2 ± 5.5**, P &lt; 0.002**</td>
<td>2.2 ± 0.4**, P &lt; 0.05**</td>
</tr>
<tr>
<td>Vehicle</td>
<td>7.2 ± 2.8</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>18.2 ± 4.2</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>28.7 ± 4.0*</td>
<td>1.8 ± 0.4*</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.01*</td>
<td>2.6 ± 0.5**</td>
</tr>
<tr>
<td></td>
<td>40.7 ± 3.5**, P &lt; 0.0001*</td>
<td>2.6 ± 0.5**</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.0007**, P &lt; 0.005**</td>
<td>P &lt; 0.0001*, P &lt; 0.01**</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD.
*Difference is significant compared with vehicle.
**Difference is significant compared with IL-1.
NS = not significant.

Statistical analysis
Results are expressed as mean ± SD of all experiments. Statistical differences were analysed by Student’s paired t-test. A value of P < 0.05 was considered statistically significant.

Results
Effects of exogenous IL-8, IL-1 and IL-8 + IL-1 on uterine contractions
The overall results of isometric uterine contractions are summarized in Table I.

In in-vivo experiments, increased frequency and amplitude were observed in rabbits treated with IL-1 (Figure 1b, P < 0.003, P < 0.0001) and IL-8 + IL-1 (Figure 1c, P < 0.0005, P < 0.0001) compared with vehicle (Figure 1d). IL-8 + IL-1 induced more potent uterine contractions with increased frequency (P < 0.002) and amplitude (P < 0.05) than only IL-1. Similar features were observed in in-vitro experiments. Treatment with IL-1 and IL-8 + IL-1 elicited significant uterine contractions with increased frequency (Figure 2b, c, P < 0.01, P < 0.0007) and amplitude (Figure 2b, c, P < 0.0001) corresponding to vehicle (Figure 2d). The uterine tissue assayed with IL-8 + IL-1 evoked more significant contraction with increased frequency (P < 0.005) and amplitude (P < 0.01) than IL-1. Investigations with IL-8 showed no significant changes in frequency and amplitude of the contractions both in vivo (Figure 1a) and in vitro (Figure 2a).

Plasma PGE\(_2\) concentration
The changes in plasma PGE\(_2\) concentration in rabbits after the administration of suppositories is shown in Figure 3. In rabbits receiving IL-8 + IL-1 suppositories, circulating PGE\(_2\) levels increased ~8-fold over vehicle (P < 0.0001). In the IL-1-treated group, PGE\(_2\) level was also increased ~2.5-fold compared with vehicle (P < 0.0001). There was no marked alteration in plasma PGE\(_2\) level when treated with IL-8.

Neutrophil influx in endometrial tissue
The number of neutrophils was increased significantly in the uterine tissue of rabbit treated with IL-8, IL-1 and IL-8 + IL-1 (P < 0.0003, P < 0.008 and P < 0.002 respectively), compared with vehicle (Figure 4). A remarkable increase in neutrophil numbers was observed in rabbits that received IL-8 + IL-1 suppositories.

Localization of neutrophils
The location and changes in density of neutrophils were identified by immunohistochemical staining (Figure 5). The stained neutrophils were located predominantly in the endomet-
IL-8 and uterine contractions

**Figure 2.** Representative traces of the responses to IL-8, IL-1 and IL-8 + IL-1 and vehicle suppositories on rabbit uterus (*in vitro*). Suppositories with IL-8 + IL-1 induced increased frequency and amplitude of uterine contractions (c, \( P < 0.0007, P < 0.0001 \)) over that of IL-1 (b, \( P < 0.01, P < 0.0001 \)), compared with vehicle (d). IL-8 could not produce contractions or increase frequency or amplitude in the rabbit uterus (a).

**Figure 3.** Prostaglandin E\(_2\) levels in plasma of rabbits treated with IL-8, IL-1 and IL-8 + IL-1, including vehicle. Investigations with suppositories containing IL-8 + IL-1 and IL-1 significantly increased prostaglandin concentration (\( P < 0.0001 \)), compared with vehicle. IL-8 had no effect on prostaglandin biosynthesis versus vehicle.

**Discussion**

It has been proposed that PGE\(_2\) plays a key role in the initiation of myometrial contraction (Kelly, 1994). IL-1 is considered as one of the most potent intermediate mediators in the synthesis of PGE\(_2\) (Romero et al., 1989b; Mitchell et al., 1990; Kelly, 1994). The regulation of IL-1 in this event has not been clearly understood.

The present study demonstrates that administration of suppositories containing IL-1 and IL-8 + IL-1 induces a potent contractile effect in rabbit uterus. Only IL-8 produced no contractile effect, but in the presence of IL-1 it promoted uterine contractions with increased frequency and amplitude. We have also shown that treatment with IL-8 + IL-1 increases the neutrophil number as well as PGE\(_2\) synthesis. The increase in neutrophil number and PGE\(_2\) synthesis were greater in the IL-8 + IL-1 group than the IL-1 only group. Previous findings suggest that the application of TNF-\(\alpha\) and IL-1\(\beta\) stimulated PGE\(_2\) production from cultured amnion, decidual and chorion cells (Mitchell et al., 1991b), whereas IL-8 did not lead to stimulation of PGE\(_2\) production in these cells (Mitchell et al., 1991a; Dudley et al., 1993), which is consistent with our observations that administration of IL-8 suppository did not induce uterine contraction in rabbits. However, IL-8 is critically involved in the process of parturition. A synergy between IL-8 and PGE\(_2\) may be operational here.

Growing evidence suggests that cytokines are present in the gestational tissue and play a major role in the triggering and perpetuation of labour (Romero et al., 1991; El Maradny et al., 1994, 1995, 1997; Kelly, 1994). Various uterine cells and cervical fibroblasts are capable of producing cytokines such as IL-8 and IL-1 (Uchiyama et al., 1992; Kelly et al., 1994). The action of IL-8 and IL-1 suggests that they interact.
According to our hypothesis, the mechanism of cross-talk between IL-8 and IL-1 is as follows. Firstly, we found that IL-8 leads to accumulation of neutrophils in rabbit endometrial tissue. The neutrophil influx and activation of neutrophils in the cervix and chorio-decidual tissue initiate a cascade of events leading to the loosening of endometrial connective tissues (Wewer et al., 1992; Kuijpers and Harlan, 1993) as well as increased permeability of the vessels (Wedmore and Williams, 1981). As a result, inflammatory cells such as macrophages infiltrate to the matrix from vessels, contributing to the synthesis of IL-1 (Kelly, 1994). Secondly, IL-8 enhances the release of other inflammatory cytokines such as IL-1 (Yu et al., 1994), which can stimulate the production of PGE₂ and enhance uterine contraction. Thirdly, there is evidence that the inflammatory cytokine IL-1 also regulates IL-8 synthesis in endometrial cells (Arici et al., 1993), which may further modulate the inflammatory reaction (Yu et al., 1994). Thus the number and existence of neutrophils in the endometrial tissue were more significantly increased in the IL-8 + IL-1-treated group.

To clarify the effect of IL-8 on myometrial contraction, we used animals which are not affected by progesterone. However, a pilot study showed that the effect of IL-8 on myometrial contraction in pregnant rabbits was similar to that in nonpregnant rabbits (data not shown). These findings suggest that IL-8 together with IL-1 enhance PGE₂ mediating uterine contraction via neutrophil transmigration at the onset of labour. IL-8 is a locally acting cytokine and possibly a small amount of IL-8 is absorbed into the circulation. However, red blood cells carry multispecific receptors that can bind a wide array of chemokines. IL-8 can be absorbed by red cells and no free IL-8 exists in the circulation (Darbone et al., 1991). Thus exogenous IL-8 might act on the endometrial tissue by simple diffusion.

In conclusion, at the onset of labour IL-8 and IL-1 concentrations increase, which triggers the production and action of PGE₂. The combination of PGE₂ and IL-8 would be a powerful stimulus for the invasion of neutrophils and their degranulation. The interaction between IL-8 and IL-1 seems to be essential for forceful uterine contraction.

Acknowledgements
This study was supported in part by Grants-in-Aid for Specific Research (no. 10671529) from the Japanese Ministry of Education and Science and the Japanese Ministry of Health and Welfare (Paediatric Research Grant no. 98–10/02).
IL-8 and uterine contractions

References


Received on June 19, 1998; accepted on November 3, 1998