Effects of neutrophil chemotactic factors on cervical ripening

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Summary: Our aim was to study the effects of infection mediated by bacterial endotoxins (lipopolysaccharides LPS) and bacterial substrate (N-formyl-methionyl-leucyl-phenylalanine FMLP) on pathological cervical ripening, and compare their effects with the normal physiological mediator interleukin-8 (IL-8).

Twenty non-pregnant rabbits were treated vaginally either by placebo, LPS, FMLP or IL-8 for 3 successive days. Water concentration was measured. Neutrophils were counted in 5 random fields of each specimen. Collagen and glycosaminoglycan concentrations were assessed histologically by using picrosirius red and alcian blue staining and optical density was calculated.

LPS, FMLP as well as IL-8 can induce cervical ripening. Water concentration was found to be significantly increased in these groups (p<0.0001). Also, significant numbers of neutrophils were found to accumulate in the connective tissue of these cervices (p<0.0001). Collagen concentration was significantly decreased in the cervices treated by LPS, FMLP and IL-8 (p<0.0001). Glycosaminoglycan concentration was significantly increased in cervices treated by LPS, FMLP as well as IL-8 (p<0.0001, 0.006 and 0.001 respectively).

LPS, FMLP and IL-8 stimulate cervical ripening in non-pregnant rabbits. This ripening was mediated through the accumulated neutrophils in the connective tissue. IL-8 has a physiological type effect to induce ripening of the cervix.

Key words: Cervical ripening; LPS; IL-8; FMLP; Neutrophil; Collagen; Glycosaminoglycan.

INTRODUCTION

The human uterine cervix is predominantly made up of collagen fibrils (1). These collagen fibrils are bound together to form bundles which are separated by a ground substance containing proteoglycans and elastin (2). These proteoglycans contain a variety of glycosaminoglycans.

Dermatan sulfate is the quantitatively dominating glycosaminoglycan, but heparan sulfate and hyaluronic acid are also found (3).

Cervical ripening is associated with significant structural and biochemical changes within the cervix. These alterations lead to remodelling and reorganization of the cervical connective tissue with the decrease in concentration of collagen and proteoglycans. The reduction in collagen concentration occurs due to increased proteases produced by fibroblasts or leukocytes (4).

It has been reported that neutrophil invasion of the cervix occurs in parallel with cervical ripening (5, 6). Neutrophil
accumulation usually occurs at the inflammatory sites (7). Accordingly, ripening of the cervix is considered as a physiological inflammatory mechanism (8). The existence of a neutrophil chemotactic factor produced by cervical tissue was suspected of being responsible for this neutrophil invasion.

It was found that the human cervix has the ability to produce large amounts of interleukin-8 (IL-8) (9). IL-8 has a potent neutrophil chemotactic effect and leads to degradation of the specific granules that contain collagenase. It may also stimulate the release of elastase from the azurophil granules (10). We found that IL-8 plays a central role in cervical maturation of normal pregnancy.

Premature cervical ripening can cause preterm delivery. Intrauterine infection is one of the most common causes of premature labor. It was suggested that ascending vaginal route is the likely source of infection (11). Bacterial endotoxins (lipopolysaccharides LPS) and bacterial substrate (N-formyl-methionyl-leucyl-phenylalanine FMLP) have also a neutrophil chemotactic effects (12, 13). We suggested that premature cervical ripening may be mediated by the chemotactic effect of bacterial substances like LPS and FMLP.

Accordingly, the aim of this research was to 1) study the effects of infection mediated by baterial endotoxins (LPS) and bacterial substrate (FMLP) on pathological cervical ripening, and 2) to compare their effects with the normal physiological mediator IL-8.

MATERIALS AND METHODS

To avoid confusion with the normal physiological changes in cervical collagen, glycosaminoglycan and neutrophil invasion which occur during pregnancy, non-pregnant rabbits were chosen as a model for this study.

Twenty non-pregnant white New Zealand female rabbits were used in this study. All the rabbits were nullipara and comparable in weights and ages. These rabbits received either the tested drugs or placebo in the form of vaginal suppositories. Single dose was applied aseptically for 3 successive days. The rabbits were divided into 4 equal groups:

Group 1 (control group): where rabbits received only the greasy material used to make the suppositories without any drug.

Group 2 (LPS group): 100 pg. lipopolysaccharide (Escherishia coli Sigma, St. Louis USA) was included in each suppository.

Group 3 (FMLP group): where 200 pg. of FMLP (Sigma Co. USA) was applied in each suppository.

Group 4 (IL-8 group): in which each suppository contained 100 ng of IL-8 (Oncogen Science).

The cervices of all animals were dissected 24 h after the last suppository. These cervices were examined clinically for consistency and mechanical dilatation. In order to estimate cervical maturation, several analyses were made.

Water concentration was measured by IM-3SCV device (Fujite Technique Company). This apparatus uses near-infrared spectrophotometric technique (measuring wavelength absorbance 1.45 nm, and reference wavelength 1.3 pm and 1.6 pm) (14). Four different points on the cervix were measured and the mean was calculated.

Histological sections were prepared and stained by H&E to study and compare the histopathological changes that occurred. Immunohistochemical staining of the rabbits’ neutrophils was carried out using anti-rabbit RT-2 monoclonal antibodies (Cedarlane Laboratories Limited, Canada) (15). The total number of neutrophils in 5 random fields of each biopsy specimen were counted, avoiding the cells which were in the blood vessels, and the mean number was calculated. Also, immunostaining of collagen type I, which is the dominating cervical collagen, was done using specific antibodies (Iwaki Co. Japan).

Relative collagen concentration was assessed by staining with picrosirius red (Sirius red P3BO Chroma-Gesell Schmid Gm bH Co.), as previously described and validated (16) as a histological method of determining the polymerized collagen concentration of tissues including that of the cervix (17). The histological analysis was performed by measuring the optical density (% polarized light transmission) from 5 random fields of the connective tissue of each biopsy and the mean optical density was calculated. An image analyzer was used for all histological measurements (Microscope: Olympus IMT-2, Video camera SIT C2400-80
and Computer analyzer system with ARGUS-100 Hamamatsu photonics). In picrosirius red staining, the greater the collagen concentration, the greater the birefringence, and hence the greater the % of light transmission (18).

Relative glycosaminoglycan concentration was assessed by the same technique as collagen but using the alcian blue staining which is specific for glycosaminoglycan (19). In alcian blue staining, the greater the light transmission, the less staining, and therefore less glycosaminoglycan concentration of the tissues (18).

Student t test was used to analyze the data. All the results were expressed as mean±SD.

RESULTS

Rabbit cervices which, treated with LPS, FMLP and IL-8, were markedly increased in size, became soft and edematous. Also, their cervical canals were easily dilated by Hégar's dilators compared to non-pregnant cervices of the control group.

Significant increase of water concentration was found in LPS, FMLP and IL-8 groups (p<0.0001) in comparison to the control group. Water concentration in LPS treated cervices was significantly higher than those treated with IL-8 and FMLP (p<0.0002 and 0.0001 respectively). Fig. 1. Also, more significant increased of water concentration was detected in IL-8 group than FMLP group (p<0.07).

Histological examination of cervices which had been stimulated with neutrophil chemotactic factors LPS, FMLP as well as IL-8 revealed accumulation of neutrophils in the connective tissue. This neutrophil accumulation was found to be mainly under the glandular epithelium and round the blood vessels (Fig. 2 a, b, c and d). Neutrophil numbers were significantly increased in these groups compared to the control group (p<0.0001) Fig. 3. Neutrophil numbers found in LPS group were significantly higher than IL-8 and FMLP groups (p < 0.0009 and 0.0001). Also, neutrophil numbers in IL-8 group were significantly greater than FMLP group (p<0.0002).

In sections of the control group stained with H&E, the connective tissue was compact and the blood vessels were small and non-dilated Fig. 4 a. While in LPS group, the connective tissue was obviously decreased and loose. The ground substance was increased and loose. The blood vessels were markedly increased and dilated Fig. 4 b. The same changes in connective tissue, ground substance and blood vessels were also observed in IL-8 group and FMLP group, but to less extent than LPS group Fig. 4 c & d. The use of specific immunohistochemical staining for collagen type I revealed that it was markedly decreased in cervices treated by LPS, FMLP as well as IL-8 Fig. 5.

Optical density (OD : % polarized light transmission) in sections stained with picrosirius red of LPS, FMLP and IL-8 groups was found to be significantly decreased compared to the control group.

* p < 0.0001 vs control group.

Fig. 1. Water concentration in non-pregnant rabbit cervices treated by LPS, IL-8 and FMLP.
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Fig. 2. — Neutrophil accumulation in the connective tissue of a) Control group; b) LPS group; c) IL-8 group; d) FMLP group. Magnification × 400.
Neutrophil numbers significantly higher than FMLP group (p<0.06).

Similar results, but more marked, were obtained when these drugs were applied to pregnant rabbits (data are not shown here).

DISCUSSION

Neutrophils play an important role in the cervical ripening. Junqueira et al. (5) and Rath et al. (6) observed that neutrophil accumulation occurs in the cervical tissue during maturation. At this time the collagenase activity was found to be increased (20). Osmers et al. (21) reported that the origin of this collagenase activity is in the neutrophils which invaded the cervix.

The ability of the cervix to produce a chemotactic factor which leads to neutrophil accumulation was intensively studied. It was found that IL-8 can be produced in large amounts by the pregnant human cervix (8). IL-8 has a dual function, to induce neutrophil migration into tissues and to cause degranulation of the specific granules that contain collagenase (10). Therefore, IL-8 may be an essential factor for cervical maturation.

Intrauterine infection is closely associated with preterm delivery (22). A high endotoxin concentration (LPS) in the cervical mucus (23) and amniotic fluid has been detected in women with preterm labor and chorioamnionitis (24). Bacterial endotoxins have a multitude of biological effects including prostaglandins and cytokines production (25). It has been shown that LPS stimulates production of IL-8 by the human cervix (7). This may also reflect the importance of the local effect of neutrophil chemotactic factors on premature cervical maturation in pathological conditions.

It has been reported that collagen concentration of the cervix at term decreases to 30% compared to non-pregnant cer-
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Fig. 4. — Histological sections of the cervixes stained by H&E showing: a) control group with compact connective tissue; b) LPS group with loose connective tissue, marked increase of dilated blood vessels; c) IL-8 group with decreased connective tissue and increased ground substance; d) FMLP group with moderate changes in connective tissue and ground substance. Magnification × 100.
Fig. 5. — Immunohistochemical staining for collagen type I, showing marked decrease of collagen type I in cervices treated with LPS, IL-8 and FMLP. a) Control group; b) LPS group; c) IL-8 group; d) FMLP group.
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Fig. 6. — Optical density (OD: % polarized light transmission) measured in sections stained by picrosirius red in the control group and cervices treated by neutrophil chemotactic factors. Decrease of OD indicates decrease of collagen concentration.

Fig. 7. — Optical density (OD: % polarized light transmission) measured in sections stained by alcan blue in control group and cervices treated by LPS, IL-8 and FMLP. Decrease of OD indicates increase of glycosaminoglycan concentration.

...vix (2). In our experiments, the cervices treated with LPS, FMLP as well as IL-8 showed significant decrease of collagen concentration. The decrease of collagen type I was markedly evident in the histological sections. It is known that collagenase produced by neutrophils selectively degrades type I collagen (28) which is the main collagen structure in the cervix.

Glycosaminoglycan concentration plays a role in the structural alteration of the cervical connective tissue during labor (27). The absolute amounts of glycosaminoglycan increases during pregnancy (28). Ripening of the cervix is associated with distinct decrease in dermatan sulfate content (29), at the same time other glycosaminoglycans such as heparan sulfate and hyaluronic acid are increased (30). Increased hyaluronic acid contributes to the loose collagenous framework at term. Hyaluronic acid itself has a high water binding capacity which may explain the increase of the water content of the human cervix at term (27).

In our experiments, we found that the total glycosaminoglycan concentration was increased in cervices treated with LPS, FMLP and IL-8. Also, the water content was found to be increased in these cervices. The increase in size and softening of the tested cervices may be partly due to this increase in water concentration.

From this research, we came to the conclusion that LPS, FMLP as well as IL-8 can induce cervical ripening in non-pregnant rabbits. This ripening is mainly mediated by neutrophils which were accumulated in the cervical tissues. The effect of FMLP to induce ripening appeared to be less than IL-8. LPS has the strongest effect on the cervices. LPS and FMLP may play an important role in the pathology of premature cervical ripening. Whereas IL-8 was observed to have a physiological effecting stimulating cervical ripening.
REFERENCES


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