Serum and Amniotic Fluid Eosinophil Cationic Protein Levels in Misoprostol Induced Pregnancies

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Abstract
The aim of this study was to determine the concentration of eosinophil cationic protein (ECP) in maternal serum (MS) and amniotic fluid (AF) at term and assess misoprostol induced changes in their mean concentrations. A total of 53 pregnant women were included in the study: 24 were in active labor, and a further 29 were not in labor and misoprostol induction was performed. The initial 3 mL of amniotic fluid was collected and processed. In all pregnant subjects, maternal blood samples were obtained from the cubital vein at the time of amniotic fluid sampling. The concentration of ECP in serum and AF was measured by chemiluminescence method with the immulite 2000 analyzer (Immulite2000® ECP, DPC Diagnostic Products Corporation 5700 West 96th Street Los Angeles, CA, USA). Misoprostol induced pregnant showed significantly higher concentrations ECP than the control pregnant women in serum. AF-ECP concentrations were not significantly different between two groups. There was no significant difference between MS and AF-ECP concentrations in control subjects. The association we encountered between labor induction with misoprostol and increased systemic production of ECP should be investigated further.

Key Words: Misoprostol; Amniotic fluid; Serum, Cervical ripening

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Introduction

Eosinophil cationic protein (ECP) was first purified from human myeloid cells. It has a variety of biological activities interacting with other immune cells and plasma proteins. The cytotoxic activity, however, is the most conspicuous. The different isoforms of ECP seem to have different biological properties with respect to cytotoxicity and the effects on fibroblasts. ECP can be measured in biological fluids, by means of sensitive immunoassays, as an indication of eosinophil turnover and activity in vivo [1].

Cervical ripening has been likened to an inflammatory reaction characterized by an influx of inflammatory cells, particularly neutrophils and monocytes into the cervical stroma [2,3]. Immediately before parturition the cervix undergoes robust changes in structure that facilitate dilatation and effacement. The ECP was always demonstrated in areas with histological signs of cervical ripening, i.e. areas in which the collagen fibrils have been replaced by unidentified amorphous substance [2,4]. Eosinophil cells actively contribute to the pathogenesis of numerous inflammatory process. Serum ECP has been increasingly used as a noninvasive inflammatory marker in asthma [5].

Cervical ripening during parturition involves biochemical and cellular changes that are similar to those occurring in tissue inflammation [2]. The induction of labor is an important part of modern obstetric practice that occurs in 15% of all pregnancies [6]. The most widely used agents for cervical ripening are the prostaglandins (PG) in a variety of forms, dosages and application routes [6-9]. Misoprostol, a synthetic PGE1 analog, is currently being evaluated for labor induction at term. Several studies have evaluated the feasibility and safety of misoprostol for induction of labor at term in women with an unfavorable cervix [8,9]. The exact mechanisms by which misoprostol increases cervical compliance, reduces cervical resistance and effects cervical ripening are not well understood.

In terms of direct application to the cervix, PGE1 induces modification of collagen and alteration of the relative concentration of glycosaminoglycans in the cervix and promotes the maturational changes of cervical ripening [6-9]. To better examine the role of ECP in parturition, we assessed whether changes in ECP concentrations occurred in maternal serum and amniotic fluid (AF) compartments after the misoprostol induction. To our knowledge, this is the first study to examine the levels of ECP in AF and serum of misoprostol induced pregnant women.

Materials and Methods

This is a prospective case-control study consisting of 2 groups of pregnant women: (1) Twenty nine pregnant women with induced misoprostol, and (2) twenty four control pregnant women matched for gestational age. None of the misoprostol induced pregnant women were in labor at the time of study. Eligible subjects were pregnant women with a singleton gestation who were admitted for labor induction. Inclusion criteria were term gestation with an unfavorable cervix (Bishop score <6), vertex presentation, intact membranes, absence of labor (<4 contractions per hour), and reassuring fetal heart rate tracing (reactive non-stress test results without decelerations). Pregnants with a history of antepartum bleeding, placenta previa, previous uterine scar, evidence of chorioamnionitis, previous use of induction agent during the index pregnancy, and contraindication to receive or known allergy to latex or prostaglandin were excluded from the study. The predominant indication for induction was post term pregnancy. Women who were undergoing labor induction received 50 μg of misoprostol (Cytotec; Ali Raif Co., Istanbul, Turkey), which was placed in the posterior fornix of the vagina every 4 hours until the onset of labor (>3 contractions in 10 minutes). Because the 50-μg tablets were not available commercially, a 200-μg tablet was cut into fourths by the hospital pharmacist. The maximum dose of misoprostol was 150 μg.

Sample Collection and ECP Measurement: Samples of amniotic fluid were collected by transvaginal route at the time of spontaneus rupture of the membranes. We used syringe. The initial 3 mL of amniotic fluid was collected and processed. Briefly, amniotic fluid samples were immediately centrifuged after collection at 1800g for 15 minutes at 4°C; the supernatants were divided into aliquots and stored at -80°C until assayed. In all pregnant subjects, maternal blood samples were drawn from the cubital vein at the time of amniotic fluid sampling. Maternal blood was obtained from the antecubital vein and collected in tubes containing ethylenediamine tetra-acetic acid and aprotinin and centrifuged at 650*g* for 15 minutes at 4°C. The plasma was divided into aliquots and stored at –80°C until assayed. The concentration of ECP in serum and AF was measured by chemiluminescence method with the immulite 2000 analyzer (Immulite2000® ECP, DPC Diagnostic Products Corporation 5700 West 96th Street Los Angeles, CA, USA). The analytical sensitivity and normal values of immunometric ECP assay were 0.2 ng/mL and 10.9-24 ng/mL, respectively.

Statistical Analyses

Statistical analyses was performed with Statistical Package for the Social Sciences for Windows (SPSS, Inc., Chicago, IL, USA). The Kruskal-Wallis analysis of variance and post-hoc multiple comparison test were performed on the data of the biochemical variables to examine differences between the groups. Demographic data of the groups were expressed as mean and standard deviation (SD). The Mann–Whitney *U*-test and chi-square test were used for statistical analysis of continuous and categorical variables, when appropriate. The results are given in the text as means ± SEM. For all comparisons, statistical significance was defined as $P < 0.05$.

Results

Obstetric characteristics of the groups in terms of age, parity, previous healthy children, gestational age at delivery and route of delivery are similar and shown in Table I. Mean MS-ECP (37.47±7.92 ng/ml) and AF-ECP (12.74±4.98 ng/ml) levels of misoprostol group were significantly different ($p<0.05$). Misoprostol induced pregnant women showed significantly higher concentrations ECP than control pregnant women in serum (Misoprostol 37.47±7.92 ng/ml; control 14.00±2.97 ng/ml). On the other hand, AF-ECP concentrations were not significantly different between two groups (Misoprostol 12.74±4.98 ng/ml; control 8.71±2.17 ng/ml). There was no significant difference between maternal serum and AF-ECP concentrations in control subjects (Figure 1).

Figure 1. Graphical representation of amniotic fluid and serum ECP levels in PGE1 and control group (Note, *: P<0.05, Mann Whitney U test).

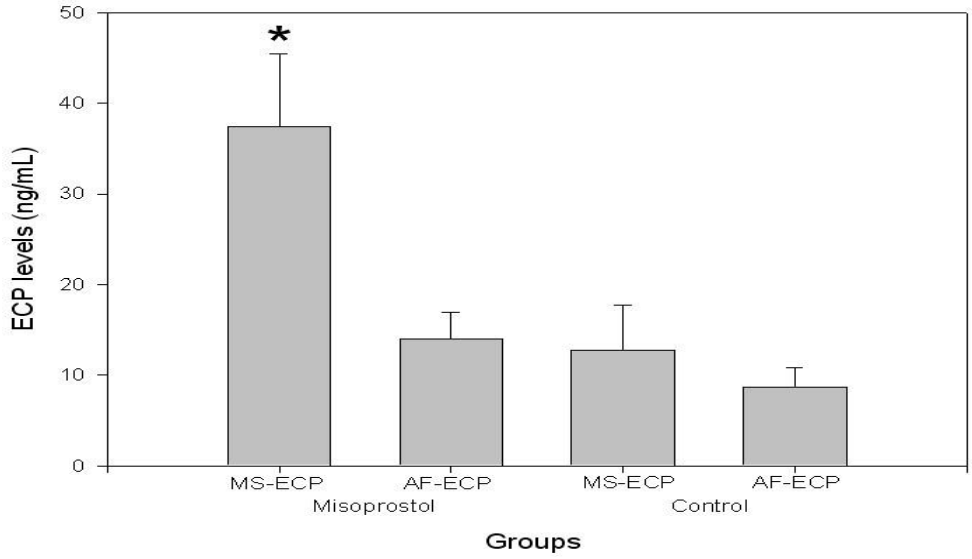

Table. 1. Demographic features of the groups

Variables	Control group (*n* = 24)	Study group (*n* = 29)	*P*-values
Age, median (range)	26.3 (18–36)	29.9 (19–40)	0.24
Gravidity, median (range)	1.7 (1–3)	2.1 (1–5)	0.42
Parity, median (range)	1 (0–3)	1 (0–4)	0.516
Previous healthy children, median (range)	1 (0–2)	1 (0–3)	0.631
Gestational week at delivery (weeks)	39.7 ± 2.7	38.1 ± 3.7	0.532
Cesarean delivery, n (%)	5 (20.8%)	9 (31%)	0.402
Birth weight of infants (g)	3300 ± 724	3710 ± 828	0.440

Values are presented as mean ± standard deviation unless otherwise stated.

Discussion

Cervical ripening shares many features in common with inflammatory-associated tissue remodeling, making it a valuable process to explore with respect to the biochemical events in extracellular matrix restructuring. The chemokines interleukin-8 and monocyte chemotactic protein-1, prostaglandins, matrix metalloproteinases and their inhibitors have all been proposed as having a role in regulating this influx and in effecting tissue remodelling [10,11]. Eosinophils have been found to contain a metalloproteinase that can degrade type I and III collagens [12], which are the two main types of collagen in the human cervix. They are involved in inflammatory responses and mediate both proinflammatory and antiinflammatory activity [13]. They contain several cytotoxic proteins including ECP and eosinophil protein X. Involvement of the eosinophil granulocyte in pregnancy and parturition in animal studies are conflicting. In rats eosinophils have been showed in the cervical tissue samples in late pregnancy. But another study have reported few eosinophils in rats during pregnancy and no increase during labour [14-16]. In the present study, maternal serum ECP level seems to reflect, although indirectly, the intensity of ongoing eosinophilic inflammation of the cervix and respond sensitively to intervention. Monitoring of serum ECP could be of utility in the follow-up of misoprostol induced pregnancies. The expression of the activated secreted forms of ECP as well as the determination of the rise of the ECP level of the serum make it very easy to follow their activity. Recent immunohistochemical studies showed that eosinophils accumulate and degranulate during the connective tissue degradation, which takes place during rat,bovine and human cervical ripening [15,17-19]. Influencing the activation of the eosinophil cell may lead to important alterations in the initiation of human parturition. Alternatively, increased serum ECP levels may have a important role in the ripening of the cervix acting on cervical structures.

Recent data have proved that eosinophil cells actively contribute to the pathogenesis of numerous [20] inflammatory diseases such as asthma and Crohn's disease [21]. Immunohistochemical studies of biopsies from organs affected by these conditions have demonstrated extracellular deposits of ECP in areas with maximum tissue destruction [17]. In the our study, maternal blood concentration of ECP was also found to be significantly higher after the misoprostol induction although the source of this increase is not known. Osmers et al have demonstrated that collagenase involved in cervical maturation during parturition is derived from polymorphonuclear leukocytes and not from fibroblasts present in the cervical

stroma. ECP may be found as a part of the collagenolytic process induced, e.g. neutrophils or chemokines, rather than one of the main causes of cervical ripening [22]. The misoprostol associated increase in maternal serum concentrations of ECP demonstrated in this study is consistent with the well characterized role of this protein in cervical inflammatory reactions. It remains to be established whether the observed increase in maternal serum ECP concentrations in misoprostol induced patients are an epiphenomenon of labour onset or is involved causally in the cervical ripening. It is possible that ECP secretion is modulated by misoprostol and it may be involved in the regulation of cervical maturational changes. Knudsen et al [17] reported that in early pregnancy PGE2 treatment did not increase the degranulation or affect the number of eosinophils in cervical biopsies. On the other hand, at the term, immediately after delivery several degranulated eosinophil granulocytes were found in after pretreatment with PGE2 and the presence of eosinophils and extracellular deposits of ECP in the PGE2 group were similar to those in women with physiological ripening.

The exact function of the ECP in cervical ripening is unclear. In many inflammatory reactions, however, a close association between deposition of eosinophil granule proteins and areas of cell destruction have been demonstrated [14,23]. The present study suggests that misoprostol may, in part, effect cervical ripening by increasing the MS-ECP levels. A better understanding of how ECP reaches the cervix and how it regulates cervical maturational changes will be important in understanding the role that misoprostol plays in the physiology of cervical ripening.

In conclusion, this study reports for the first time the participation of ECP in cervical ripening at term in misoprostol induced pregnant women. Women who delivered spontaneously showed low levels of MS-ECP and AF-ECP than misoprostol induced deliveries. ECP could participate by means or these metalloproteinases action in the decrease in collagen concentration which is seen in maternal serum from misoprostol induced women. Our results supports the hypothesis that misoprostol induced cervical ripening has many similarities to an inflammatory process. However, further longitudinal studies are required to establish the role of serum ECP measurement in the cervical rippening in misoprostol induced pregnant patients.

Disclosure: After publication of this manuscript, three authors declared a conflict and wanted to witdrawn their names. Regarding to this conflict, editorial board removed their names on April 12, 2013.

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