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Differential kinetics of serum and cervical insulin-like growth factor-binding protein-1 during mifepristone–misoprostol-induced medical termination of early pregnancy

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The kinetics of cervical and circulating phosphoisoforms of insulin-like growth factor-binding protein-1 (IGFBP-1) in normal and pathological early pregnancy are not well known. We investigated the profiles of IGFBP-1 in serum and in cervical secretion during medical termination of early pregnancy. Sixteen women requesting termination of pregnancy, with <63 days of amenorrhoea, received 200 mg of mifepristone on day 0, followed by either oral or vaginal administration of 0.8 mg of misoprostol on day 2. Serum and cervical swab samples, collected up to 6 weeks following the beginning of the treatment, were analysed for IGFBP-1 using two immunoenzymometric assays recognizing different patterns of IGFBP-1 phosphoisoforms. Serum mifepristone was also assayed. In the cervical samples, IGFBP-1 concentration, measured with both assays, increased substantially 2 days following administration of mifepristone. At 3 h after administration of misoprostol, IGFBP-1 had further increased several-fold in the cervix, but the increase was more pronounced as measured by the assay with preference for the amniotic fluid isoforms of IGFBP-1. A strong negative correlation was found between the time to abortion and the increase in cervical IGFBP-1 after administration of misoprostol, as measured by the assay preferring the phosphorylated isoforms of IGFBP-1. At 6 weeks, IGFBP-1 in the cervix had decreased to lower than pre-treatment levels, as measured by both assays. In serum, both assays showed a significant increase in IGFBP-1 concentrations after administration of mifepristone, and the highest values were measured on day 2, already before misoprostol administration. Thus, the kinetics of circulating and cervical IGFBP-1 differed from each other, indicating different sources and regulation of serum and cervical IGFBP-1.

Key words: early pregnancy/IGFBP-1/medical abortion/mifepristone/misoprostol

Introduction

Insulin-like growth factor-binding protein-1 (IGFBP-1) is produced mainly by the liver and decidualized endometrium (Rutanen *et al*., 1985; Julkunen *et al*., 1988) but high concentrations (100–1000-fold higher than those in serum) of IGFBP-1 are also found in amniotic fluid (Rutanen *et al*., 1985; Julkunen *et al*., 1988). IGFBP-1 in amniotic fluid differs from that in decidua in its phosphorylation status (Martina *et al*., 1997; Nuutila *et al*., 1999). Amniotic fluid contains non-phosphorylated and less phosphorylated isoforms of IGFBP-1, whereas decidua contains phosphorylated isoforms including a highly phosphorylated isoform not present in amniotic fluid (Rutanen *et al*., 1982; Martina *et al*., 1997). The phosphorylation status of IGFBP-1 affects its ability to bind insulin-like growth factors (IGF); the phosphorylated forms have higher IGF-binding affinity compared with less and non-phosphorylated isoforms (Jones *et al*., 1991). Both in decidua and in amniotic fluid the degree of IGFBP-1 phosphorylation increases from early pregnancy to late pregnancy (Koistinen *et al*., 1993).

Assessment of the different profiles of IGFBP-1 phosphoisoforms is utilized in clinical practice. The detection of amniotic fluid isoforms of IGFBP-1 in the cervix is used to diagnose rupture of fetal membranes (actim™ PROM test; Medix Biochemica, Finland) (Rutanen *et al*., 1996). Increased levels of decidua-derived, phosphorylated isoforms of IGFBP-1 in cervical secretion have been shown to predict cervical ripening in term pregnancy (Nuutila *et al*., 1999). In addition, an increase in the levels of phosphorylated isoforms of IGFBP-1 in the cervix indicates a risk of preterm delivery (Kekki *et al*., 2001; Lembet *et al*., 2002).

The combination of bacterial vaginosis and increased levels of cervical phosphorylated isoforms of IGFBP-1—probably a marker of tissue damage at the chorio-decidual interface—at 10–17 weeks of gestation predicts an increased risk of infectious morbidity later in pregnancy (Kekki *et al*., 1999). However, to the best of our knowledge, the cervical release of IGFBP-1 in normal or pathological early pregnancy has not been studied elsewhere. Moreover, the regulation and source of circulating IGFBP-1 in early pregnancy is poorly understood.

In medical termination of pregnancy, administration of mifepristone results in uterine contractions and decidual bleeding. Subsequent administration of prostaglandin enhances uterine contractions and results in expulsion of fetal material. Medical termination of pregnancy thus greatly resembles spontaneous miscarriage. We

therefore evaluated the kinetics of IGFBP-1 both in serum and in cervical secretion during medical termination of early pregnancy with mifepristone and misoprostol.

Materials and methods

Subjects

This study was carried out along with a World Health Organization double-blind multicentre trial (von Hertzen *et al*., 2003) comparing the efficacy of the route and duration of misoprostol administration in combination with mifepristone for medical abortion. The eligibility criteria used were the same as described in a previous study (World Health Organization, 2000). Only women with single intrauterine pregnancies, confirmed by ultrasonography, were included.

The study group comprised 16 healthy women (age range 19–40, mean 31 years) requesting medical termination of an unwanted pregnancy, with <63 days of amenorrhoea, at the Department of Obstetrics and Gynaecology, Helsinki University Central Hospital, Helsinki, Finland. The study was approved by the local ethics committee. Each subject gave informed consent prior to participation in the study.

In the efficacy study the women were randomized to one of three treatment groups. All women were given an oral dose of 200 mg mifepristone on day 0 of the study. The study was placebo-controlled regarding the route of administration of misoprostol on day 2, and the continuation of misoprostol treatment on days 3–9. On day 2 of the study (36–48 h after the administration of mifepristone), six women received 0.8 mg of misoprostol orally, while 10 women received it vaginally. After administration of misoprostol, the women were observed for 3 h in the hospital. They were asked to note the onset of bleeding and the time of expulsion of fetal material.

Starting on day 3, 11 women continued with 0.4 mg of oral misoprostol twice daily for 7 days.

The women returned for follow-up visits 2 weeks and 6 weeks after beginning the treatment. Final assessment of complete abortion was carried out at the 6 week visit.

Samples of serum and cervical secretion were obtained six times: sample I on day 0, just prior to administration of mifepristone; sample II on day 2, prior to administration of misoprostol; sample III on day 2, ~3 h after misoprostol; sample IV on day 3, prior to the first home misoprostol or placebo; sample V at 2 weeks and sample VI at 6 weeks after beginning the treatment. The cervical samples were taken with Dacron swabs in speculum examination, as previously described (Nuutila *et al*., 1999; Kekki *et al*., 2001). A total of 12 women noted the exact time of abortion.

Based partly on the same subject material, we have previously reported the effects of the route and duration of misoprostol administration on the disappearance of serum hCG and progesterone (Honkanen *et al*., 2002).

Assays

Concentrations of IGFBP-1 were measured by using two immunoenzymometric assays (Rutanen *et al*., 1993; Nuutila *et al*., 1999), based on monoclonal antibodies (Rutanen *et al*., 1988).

Monoclonal antibody (Mab) 6303 (Medix Biochemica, Finland) detects all phosphorylated isoforms, including the highly phosphorylated isoform produced by decidua that is not present in amniotic fluid (Westwood *et al*., 1994; Martina *et al*., 1997). The assay using Mab 6303 in combination with Mab 6301 has been described previously (Nuutila *et al*., 1999). The detection limit of the assay was 0.25 µg/l and the intra- and inter-assay coefficients of variation (CV) were 4.6 and 6.4% respectively.

Mab 6305 (Medix Biochemica) detects the non-phosphorylated and the less phosphorylated isoforms present in amniotic fluid (Westwood *et al*., 1994; Martina *et al*., 1997), but does not recognize the highly phosphorylated isoform of IGFBP-1 (Westwood *et al*., 1994). The assay using Mab 6305 has been described elsewhere (Rutanen *et al*., 1993). The detection limit of the assay was 0.4 µg/l and the intra- and inter-assay coefficients of variation were 3.4 and 7.4% respectively. All samples from an individual subject were measured in the same assay.

Serum mifepristone was assayed as described previously (Heikinheimo *et al*., 1986).

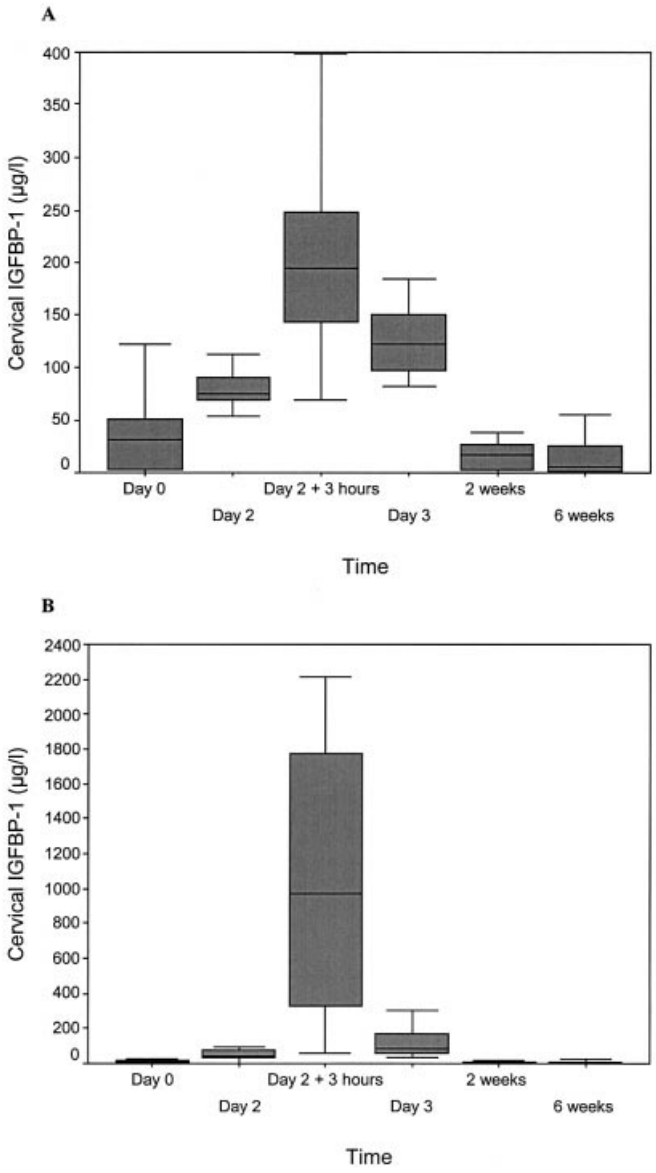

Figure 1. (**A**) Concentrations (minimum, 25th percentile, median, 75th percentile and maximum) of insulin-like growth factor-binding protein-1 (IGFBP-1) in cervical secretion measured by the assay preferring the phosphorylated isoforms. (**B**) Concentrations (minimum, 25th percentile, median, 75th percentile and maximum) of IGFBP-1 in cervical secretion measured by the assay preferring the amniotic fluid isoforms.

Statistical methods

Skewness of the IGFBP-1 values was corrected by logarithmic transformation before analysis of variance for repeated measures to analyse the longitudinal changes in IGFBP-1 levels in the treatment groups. Data on IGFBP-1 concentrations are presented as medians, and as 25th and 75th percentiles. Comparison of two groups was carried out by the Mann–Whitney *U*-test. Comparison of concentrations before treatment and at 6 weeks was carried out by Wilcoxon's signed rank test. Correlations between variables were assessed by calculating Spearman's coefficient of correlation (r). These analyses were performed using SPSS 8.0 for Windows. $P < 0.05$ was considered statistically significant.

Results

Clinical course

All 16 women had complete abortion. A total of nine women started to bleed before administration of misoprostol. The median time to

abortion after administration of misoprostol in the 12 subjects who noticed the expulsion of fetal material was 210 min (range 135–719). At 2 weeks, 12 women were still bleeding.

Cervical IGFBP-1

The profiles of IGFBP-1 in cervical secretion were similar in the three treatment groups (vaginal or oral misoprostol on day 2, continuation of oral misoprostol for 7 days or not); thus the data on all subjects were combined for the analyses. The data on these 16 subjects are presented in Figure 1A and B.

The median levels of IGFBP-1 in cervical secretion at the consecutive time-points were 27.0, 73.0, 196.0, 118.0, 17.5 and 5.2 µg/l, as measured by the assay preferring the phosphorylated isoforms (Figure 1A). On day 2, prior to administration of misoprostol, the median level in cervical secretion was 2.7×pre-treatment median (PTM) ($P < 0.05$). At 3 h after administration of misoprostol, it had further increased, being 7.3×PTM. At day 3, the median level of cervical IGFBP-1 had decreased to 4.4×PTM. Two weeks later, it was 0.6×PTM, and at 6 weeks, it was 0.2×PTM ($P < 0.05$ versus PTM).

The median levels of IGFBP-1 in cervical secretion were lower as measured by the assay preferring the amniotic fluid isoforms at each time-point, except on day 2, 3 h after administration of misoprostol, when the median level was 4.6 times higher by the assay preferring the amniotic fluid isoforms. The median levels of IGFBP-1 in cervical secretion at the consecutive time-points were 10.0, 45.0, 911.0, 94.5, 8.3 and 2.4 µg/l (Figure 1B). On day 2, prior to administration of misoprostol, the median level of cervical IGFBP-1 was 4.5×PTM ($P < 0.05$), and at 3 h after administration of misoprostol it was at its highest, 91.1×PTM. By day 3, the median level of cervical IGFBP-1 had fallen to 9.5×PTM. At 2 weeks, the median level was 0.8×PTM, and at 6 weeks, 0.2×PTM ($P < 0.05$ versus PTM).

A strong correlation was found between the IGFBP-1 values measured by the two assays in cervical secretion before any medication ($r = 0.98$, $P < 0.01$).

No difference was found between subjects receiving misoprostol orally and those receiving it vaginally in the percentage increase in IGFBP-1 levels at day 2 measured by either assay.

Women who started to bleed before misoprostol administration did not differ from those who did not, as regards the levels of cervical IGFBP-1 on day 2, and the percentage changes in IGFBP-1 (day 0 versus day 2), measured by either assay. Similarly, at 2 weeks, the cervical secretion levels of IGFBP-1 in the women who were still bleeding did not differ from those who had stopped bleeding.

The time to abortion correlated inversely with the increase in cervical IGFBP-1 after administration of misoprostol on day 2, measured by the assay preferring the phosphorylated isoforms ($r = –0.73$, $P < 0.05$), whereas it did not correlate with the concentration measured by the assay preferring the amniotic fluid isoforms.

Serum IGFBP-1

The profiles of IGFBP-1 in serum, as measured by the two assays, were similar in the three treatment groups, thus the data on all subjects were combined for the analyses. The data on these 16 subjects are presented in Figure 2A and B.

The median levels of IGFBP-1 in serum at the consecutive time-points were 86.0, 232.5, 175.0, 147.0, 71.0 and 64.0 µg/l (Figure 2A), as measured by the assay preferring the phosphorylated isoforms. On day 2, prior to administration of misoprostol, the median level of serum IGFBP-1 was 2.7×PTM ($P < 0.001$). At 3 h after administration of misoprostol on day 2, it had slightly decreased, being 2.0×PTM. By day 3, the median level of IGFBP-1 had further decreased to 1.7×PTM. At 2 weeks, the median had decreased to 0.8×PTM, and at 6 weeks, to 0.7×PTM (NS versus PTM).

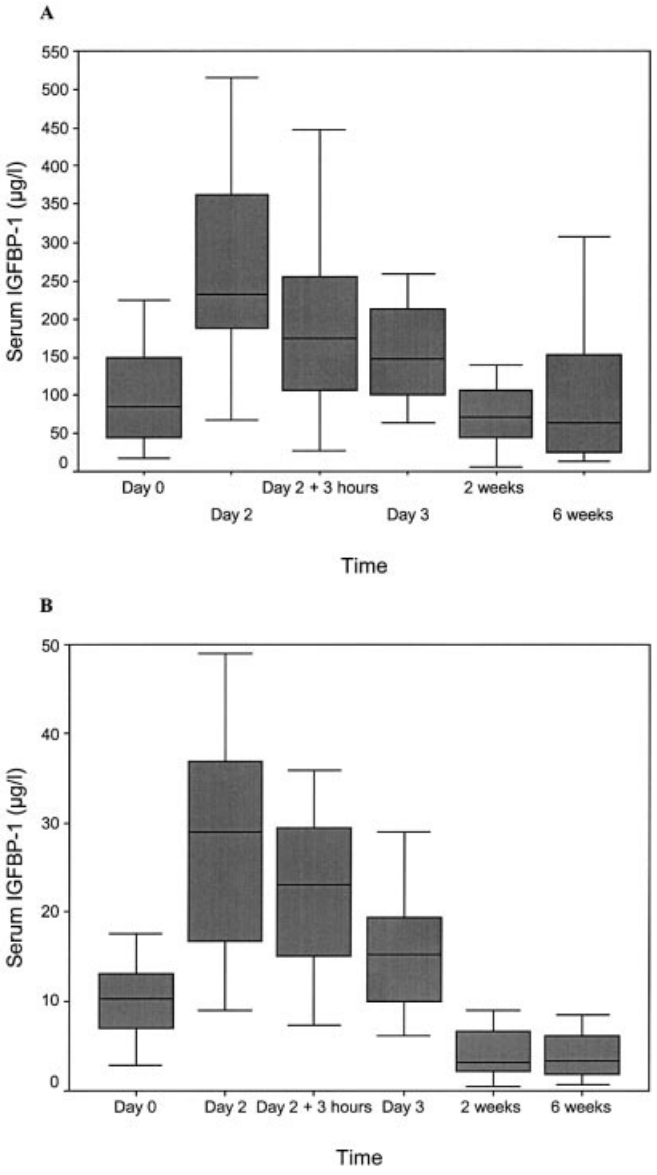

Figure 2. (**A**) Serum levels (minimum, 25th percentile, median, 75th percentile and maximum) of insulin-like growth factor-binding protein-1 (IGFBP-1) measured by the assay preferring the phosphorylated isoforms. (**B**) Serum levels (minimum, 25th percentile, median, 75th percentile and maximum) of IGFBP-1 measured by the assay preferring the amniotic fluid isoforms.

A similar trend was noted in the serum kinetics of IGFBP-1 as measured by the assay preferring the amniotic fluid isoforms (Figure 2B). The median levels at consecutive time-points were 10.3, 29.0, 23.0, 15.3, 3.1 and 3.3 µg/l. On day 2, prior to administration of misoprostol, the median serum IGFBP-1 level was 2.8×PTM ($P < 0.001$). At 3 h after administration of misoprostol on day 2, it had declined slightly, to 2.2×pre-treatment median. By day 3, the median level had decreased to 1.5×PTM. At 2 and 6 weeks, it was 0.3×PTM ($P < 0.05$ versus PTM).

There was a significant correlation between serum IGFBP-1 measured by the two assays before any medication ($r = 0.61$, $P = 0.013$), and 6 weeks later ($r = 0.86$, $P < 0.01$).

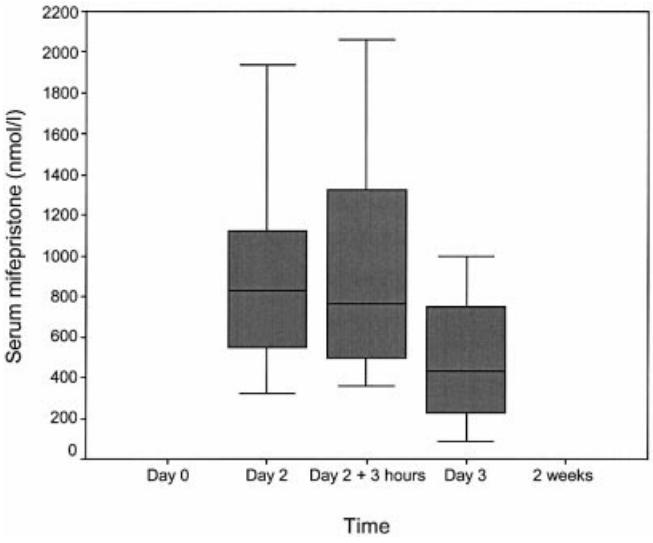

Figure 3. Serum levels (minimum, 25th percentile, median, 75th percentile and maximum) of mifepristone.

Serum mifepristone

Figure 3 shows the serum levels of mifepristone. There was a significant negative correlation between serum IGFBP-1 level on day 2, 3 h after misoprostol, measured by the assay preferring the amniotic fluid isoforms, and the peak mifepristone level on day 2 ($r = -0.58$, $P = 0.02$).

The percentage changes in serum and cervical IGFBP-1 from day 0 to day 2, measured by either assay, did not correlate with the peak mifepristone level measured on day 2. Moreover, the percentage changes in serum IGFBP-1, measured by either assay, and in serum mifepristone from day 2 (before misoprostol, or 3 h after misoprostol) to day 3 did not correlate.

Discussion

In the present study we investigated the kinetics of IGFBP-1 both in serum and in cervical swab samples during medical termination of early pregnancy, using mifepristone followed by oral or vaginal misoprostol. The IGFBP-1 was quantified using two immunoenzymometric assays based on different monoclonal antibodies preferring different patterns of IGFBP-1 phosphoisoforms (Rutanen *et al*., 1993; Nuutila *et al*., 1999). As shown previously (Nuutila *et al*., 1999), one of the assays gives higher values at instances where non- and less phosphorylated isoforms of IGFBP-1—such as in the presence of amniotic fluid—predominate. In contrast, the other assay (Rutanen *et al*., 1993) prefers phosphorylated isoforms of IGFBP-1 present in decidua (Westwood *et al*., 1994; Martina *et al*., 1997).

In agreement with the results of previous studies in women with term pregnancy (Nuutila *et al*., 1999), the levels of IGFBP-1 measured by the assay preferring the phosphorylated isoforms exceeded those of IGFBP-1 measured by the assay preferring the amniotic fluid isoforms, in samples of cervical secretion of early first trimester pregnancy. The median concentrations of IGFBP-1 in cervical samples measured by both assays increased following mifepristone administration. This increase was rapidly and significantly augmented by administration of misoprostol, but no such change was noted in serum. The median values of IGFBP-1 in cervical secretion measured by both assays also declined rapidly, reaching pre-treatment levels at 2 weeks, and had decreased to below pre-treatment levels by 6 weeks. The kinetics of IGFBP-1 in serum differed from that in cervical secretion, and mirrored that of serum mifepristone. Thus, in serum, the highest concentrations of IGFBP-1 measured by both assays were reached after mifepristone but before misoprostol administration.

The clinical course of medical termination of early pregnancy with mifepristone and misoprostol is well known. Administration of mifepristone results in uterine contractions, and bleeding in 22–41% of women within 2 days (De Nonno *et al*., 2000; World Health Organization, 2001). Expulsion of the products of conception occurs in almost 50% of subjects within the first 3–4 h after misoprostol administration (Spitz *et al*., 1998; World Health Organization, 2000). In our study, 56% of the women started to bleed following mifepristone, and the median time to expulsion of the products of conception was 3.5 h after misoprostol administration.

The rapid increase in cervical IGFBP-1 after administration of mifepristone is likely to originate from detachment of the decidua. *In vitro*, in cultures of endometrial stromal cells, progestins stimulate IGFBP-1 production (Bell *et al*., 1991; Tseng *et al*., 1992; Gao *et al*., 1994). However, in long-term cultures, progestin withdrawal further increases production of IGFBP-1 (Bell *et al*., 1991), and IGFBP-1 mRNA levels have been observed to be transiently increased in 2–3 days after replacing progestin with the antiprogestin mifepristone (Tseng *et al*., 1992). Also, *in vivo*, administration of mifepristone in the early luteal phase increases immunostaining of IGFBP-1 in human endometrium (Qiu *et al*., 2002). Thus, the observed increase of IGFBP-1 in cervical secretion might also reflect increased decidual synthesis of IGFBP-1 as a response to functional progesterone withdrawal caused by mifepristone. This is supported by the fact that the concentrations of IGFBP-1 were not different in subjects who reported uterine bleeding prior to misoprostol administration, and those who did not.

On day 2, following administration of misoprostol, IGFBP-1 levels increased markedly in cervical secretion. A significant inverse correlation was found between the time to abortion and the increase in cervical IGFBP-1 on day 2 measured by the assay preferring the phosphorylated isoforms. This is likely to reflect the increased uterine sensitivity to exogenous prostaglandin—extensive decidual damage as evidenced by the increase in cervical IGFBP-1 correlates with the increased uterine contractility and rapid expulsion of fetal material. However, at this time-point, the increase in IGFBP-1 measured by the assay preferring the amniotic fluid isoforms was more pronounced, most likely reflecting amniotic fluid leakage (Nuutila *et al*., 1999). By day 3 the concentrations of IGFBP-1 had decreased significantly, and at 6 weeks, to less than pre-treatment levels. We thus conclude that the kinetics of IGFBP-1 isoforms in cervical secretion are in accordance with the well-characterized clinical course of medical abortion.

The regulation of circulating IGFBP-1 during pregnancy is poorly understood. Depending on the assay, either an increase or unchanged levels of IGFBP-1 throughout pregnancy have been reported (Westwood *et al*., 1994; Hills *et al*., 1996). The highly phosphorylated isoform of IGFBP-1 predominates throughout pregnancy (Westwood *et al*., 1994). However, the overall phosphorylation status of circulating IGFBP-1 changes during pregnancy, with the appearance of non- and less phosphorylated isoforms of IGFBP-1 (Westwood *et al*., 1994). Similarly, in our study, the serum concentrations of IGFBP-1, as measured by the assay preferring the phosphorylated isoforms of IGFBP-1, were 8-fold higher before treatment than those measured by the assay preferring the amniotic fluid isoforms. Following termination of pregnancy, the decrease in the non- and less phosphorylated isoforms of IGFBP-1 was more pronounced, thus at 2 and 6 weeks the corresponding ratios were 23:1 and 20:1, respectively. Roughly similar ratios of 2:1 in late first trimester and 11:1 in non-pregnant women have been reported previously (Westwood *et al*., 1994).

A substantial increase in IGFBP-1 in serum was evident 2 days after administration of mifepristone. The kinetics of IGFBP-1 mirrored the circulating levels of mifepristone, yet the increase in IGFBP-1 measured by either assay did not correlate with the peak level of serum mifepristone. Serum IGFBP-1 levels have been shown to fall immediately after administration of mifepristone in first trimester medical abortion, and to rise to higher than pre-treatment levels in 2 days (Olajide *et al*., 1989). Due to a paucity of initial sampling after administration of mifepristone, we have possibly missed an initial decrease in serum levels of IGFBP-1.

The mechanism by which mifepristone increases circulating IGFBP-1 levels remains enigmatic. Regeneration of decidual cells has previously been proposed as a source of increased serum IGFBP-1 (Olajide *et al*., 1989). However, regeneration of decidua in the presence of high levels of antiprogestin is unlikely. Moreover, the serum levels of IGFBP-1, measured by the assay preferring the phosphorylated isoforms, exceeded those in cervical secretion on the morning of day 2, suggesting that decidual synthesis is an unlikely source of increased serum IGFBP-1.

The effect of progesterone on circulating concentrations of IGFBP-1 is poorly understood; thus the effects of mifepristone on serum IGFBP-1 may not necessarily represent antiprogestagenic actions of mifepristone. *In vitro*, glucocorticoids stimulate IGFBP-1 mRNA expression in hepatoma cells (Orlowski *et al*., 1990). However, *in vivo* administration of dexamethasone to male volunteers suppresses serum levels of IGFBP-1 (Miell *et al*., 1993). As mifepristone is also an antiglucocorticoid, the increase in circulating IGFBP-1 might also reflect systemic antiglucocorticoid effects of mifepristone.

We conclude that the kinetics of IGFBP-1 in cervical secretion are in line with the clinical course of medical abortion. Moreover, the increase in IGFBP-1 measured by the assay preferring the phosphorylated isoforms was inversely and significantly correlated with the time to abortion. The kinetics of circulating IGFBP-1 differed from those in the cervix, indicating different regulation and most likely different sources of serum and cervical IGFBP-1. We thus speculate that the increase in serum IGFBP-1 is a result of a systemic action of mifepristone—possibly an antiprogestin and/or antiglucocorticoid action.

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