Human Reproduction vol.12 no.4 pp.815–822, 1997

Distribution of the A and B forms of the progesterone receptor messenger ribonucleic acid and protein in uterine leiomyomata and adjacent myometrium

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The two forms of the progesterone receptor, PR-A and PR-B, are independently regulated at the transcriptional level, and show distinct responses to progesterone antagonists. We were interested in possible differences in the PR-A to PR-B ratio between uterine myometrium and leiomyomata (fibroid), that might influence the response of fibroids to progesterone agonists and antagonists, and thus have consequences for the treatment of this condition. Fibroid and adjacent normal myometrium were obtained from 11 women undergoing hysterectomy. Immunohistochemistry using a monoclonal antibody which recognizes both PR-A and PR-B showed exclusively nuclear staining, and this was stronger in the leiomyomata than in adjacent myometrium. An antibody specific for PR-B gave fainter staining of both tissues. Western blotting confirmed a higher concentration of PR in leiomyomata than myometrium in eight out of 11 cases. In all cases both forms were present, with a consistent dominance of PR-A over PR-B. However an RNase protection assay showed that there was no difference between the concentrations of mRNA encoding PR-A and PR-B, or between the mRNA concentrations in leiomyomata and normal myometrium. We conclude that the observed differences between the levels of immunoreactive PR in leiomyomata and myometrium may result from post-translational control, and support the use of progesterone antagonists in the treatment of leiomyomata.

Key words: progesterone receptor/fibroid/protein determination/messenger RNA/RU 486

Introduction

Uterine leiomyomata (fibroids) are common benign tumours of uterine smooth muscle cells. Although not malignant, they grow rapidly and are the most common indication for hysterectomy. The underlying cause of leiomyomata is not known but it is clear that ovarian steroid hormones play a role in their growth. Traditionally, medical therapies have focused on antagonizing the action of oestrogens on the leiomyomata. For example, administration of gonadotrophin-releasing hormone (GnRH) agonists suppresses pituitary gonadotrophin production and ovarian oestrogen secretion resulting in shrinkage of the leiomyomata after 3 months' treatment (Filicori *et al.*, 1983; Friedman *et al.*, 1987; West *et al.*, 1987). Consistent with these findings is an elevation in the number of oestrogen receptors in leiomyomata compared to the neighbouring myometrium (Brandon *et al.*, 1995).

However, recent reports suggest that progesterone is of significance in the growth of leiomyomata (Smith, 1993), and might act as a growth promotor as in breast glandular tissue. Kawaguchi *et al.* (1989) showed that the mitotic activity in fibroids is higher in the secretory phase than in the proliferative phase of the cycle. Myomas from post-menopausal women receiving oestrogen replacement therapy demonstrated low proliferative activity, whereas those from women receiving combined oestrogen + progestin replacement demonstrated a proliferative index equal to that observed in pre-menopausal women (Lamminen *et al.*, 1992). Myomas and myometrial cells cultured in the presence of oestrogen and progesterone appeared more active under the electron microscope than cells cultured in oestrogen-containing or control medium (Kawaguchi *et al.*, 1985). Some of the biological effects of steroid hormones are mediated through polypeptide growth factors (Nelson *et al.*, 1992). While this has not been demonstrated for progesterone, the level of mRNA encoding the angiogenic vascular endothelial growth factor (VEGF) is higher in the secretory phase than in the proliferative phase of normal myometrium (Harrison-Woolrych *et al.*, 1995). Also in the secretory phase of the cycle the level of mRNA encoding epidermal growth factor (EGF) is higher in leiomyomata than in normal myometrium (Harrison-Woolrych *et al.*, 1994).

Further support for the role of progesterone in fibroid growth comes from the study by Murphy *et al.* (1993). These authors showed that treatment for 3 months before surgery with RU 486, an antiprogestin, decreased the size of the fibroid by 49%. The efficacy of anti-oestrogenic therapy, such as treatment with GnRH agonists, could therefore be due to their suppression of ovulation which prevents the progesterone peak in the luteal phase. This is supported by the observation of Friedman *et al.* (1990) who showed that women who were treated with a combination of GnRH agonists and progestin did not show any significant regression of their leiomyomata.

The progesterone receptor (PR) is unusual in the nuclear receptor superfamily in that two isoforms of receptor exist. Receptor A (PR-A) is 94 kDa and receptor B (PR-B) is 120 kDa (Horwitz and Alexander, 1983). PR-A and PR-B result from translation of two different mRNA populations which arise by transcription from alternative initiation sites within the same gene. The transcription of the two forms is controlled by two different promoters that are both oestrogen-

induced (Kastner *et al.*, 1990a). *In vitro* these promoters are regulated independently (Gronemeyer *et al.*, 1991) and this suggests that they could be regulated in a tissue-specific manner. The exact role of each form is unclear at present; however, in the presence of agonist, PR-A and PR-B are functionally different *in vitro*. Also, PR isoforms have differential target gene specificity and may interact differently with a given promoter (Tora *et al.*, 1988; Kastner *et al.*, 1990b). For example PR-A is able to activate the ovalbumin promoter, whilst PR-B is not; however PR-B is able to activate the mouse mammary tumour virus (MMTV) promoter more efficiently than PR-A (Meyer *et al.*, 1990). Since there is differential gene regulation by PR-A and PR-B, tissue-specific gene expression may be established by altering the balance between the PR-A and PR-B isoforms. This in turn could lead to variations in tissue-specific responses to progestins (Turcotte *et al.*, 1991).

The situation is even more complex in the presence of progesterone antagonists since in this case PR-B behaves as a partial agonist (Meyer *et al.*, 1990). Antagonist binding to PR-B inappropriately activates transcription by a mechanism that does not require the progesterone response element, so that a gene that is not normally a progesterone target could be aberrantly activated (Tung *et al.*, 1993). PR-A also binds antagonist; however, this complex does not promote transcription. Furthermore PR-A in the presence of antagonist is able to act as a dominant negative repressor and blocks the agonist-like action of activated PR-B. (After binding of ligand the progesterone receptor can homo- or hetero-dimerize prior to gene activation.) Thus the final cellular response would depend on the ratio of the two forms (Vegeto *et al.*, 1993).

PR-A and PR-B have been reported to be present in chicken oviduct at equimolar concentrations (Gronemeyer *et al.*, 1987). However PR-B is the only form described in the rabbit genital tract (Loosfelt *et al.*, 1984). In human cell cultures equimolar concentrations for both forms have been reported in the breast cancer cell line T47D (Horwitz and Alexander, 1983) and endometrial cancer cells (Feil *et al.*, 1988). In human tissues, a predominance of PR-A in leiomyomata compared with myometrium has been suggested but with a limited number of samples (Brandon *et al.*, 1993). Since progestagen antagonist therapy offers several new therapeutic opportunities, it is of great importance to characterize the receptors which will interact with these drugs (Van Look, 1995). We therefore decided to examine the concentration of PR in fibroids and in adjacent normal myometrium and to determine the ratio of the two forms at both the protein and mRNA levels.

Materials and methods

Subjects

Eleven Caucasian women aged 35–53 years were included in this study. All had regular menstrual cycles and had taken no hormonal medication for at least 3 months prior to the study. They were undergoing hysterectomy for symptomatic leiomyomata at Addenbrookes Hospital NHS Trust, Cambridge, UK. Written informed consent was obtained from each patient and the study approved by the Ethical Committee of the Trust. Each uterus was examined by a pathologist in order to exclude malignant change and the endometrium from each uterus was dated (Noyes *et al.*, 1950).

Five pairs of matching leiomyomata and adjacent myometrium were obtained in the proliferative phase, and six pairs in the secretory phase. Immediately on collection, tissue specimens consisting of about 0.5 g of fibroid and 0.5 g of normal adjacent myometrium were rinsed with phosphate buffered saline (PBS) to remove excess blood, flash-frozen in liquid nitrogen and stored at –70°C until used for protein and RNA extractions. A portion of tissue containing the boundary between the myometrium and the fibroid was fixed in formalin and then embedded in paraffin wax for immunohistochemistry.

Materials

Mouse monoclonal antibody against PR (NCL-PgR) was purchased from Novocastra, Euro-Path Ltd, Bude, UK. Mouse monoclonal antibody against PR-B (B30) was obtained from K. Horwitz (Colorado University, CO, USA) (Estes *et al.*, 1987). Biotin-streptavidin-peroxidase complex (Vectastain Elite) was purchased from Vector Laboratories (Peterborough, UK). Mouse immunoglobulins (irrelevant antibody), goat serum and biotinylated goat anti-mouse immunoglobulin were from Dako (High Wycombe, UK). Aprotinin, Leupeptin, Pepstatin A, Bacitracin and phenylmethylsulphonyl fluoride (PMSF) were obtained from Sigma (Poole, Dorset, UK) and Immobilon (PVDF) from Millipore (Bedford, MA, USA). Enhanced chemiluminescence system (ECL-kit), biotinylated molecular weight markers were from Amersham International (Little Chalfont, Buckinghamshire, UK). Super Taq polymerase, reverse transcriptase and human placental ribonuclease inhibitor RNAsin were purchased from HT Biotechnology (Cambridge, UK). Neutralized deoxynucleotide triphosphates (dNTPs) and oligo-deoxythymidine (oligo dT) were from Pharmacia Ltd (Milton Keynes, UK). PCR Script SK(+) Cloning kit was from Stratagene (La Jolla, CA, USA), Maxiscript and RPA II kits were from Ambion (Austin, TX, USA). Guanidinium thiocyanate was obtained from Gibco-BRL (Uxbridge, UK).

Immunohistochemistry

From the formalin-fixed embedded tissue, 10 μm sections were cut and mounted onto slides coated with 2% APES (Sigma, St Louis, MO, USA). Three consecutive sections for each patient were dewaxed in xylene and rehydrated through an alcohol series to tap water. Sections were placed in boiling sodium citrate (0.01 M, pH 6) in a stainless steel pressure cooker and incubated 1 min at maximum pressure. Endogenous peroxidase activity was destroyed by a 10 min incubation with 3% hydrogen peroxide at 37°C. Blocking of non-specific binding was performed with 10% goat serum in PBS for 1 h. PR was localized using a mouse monoclonal antibody (NCL-PGR) which does not distinguish between PR-A and PR-B (used at 1 in 20 dilution). PR-B was specifically localized using the antibody B30 (mouse monoclonal IgG1, concentration 175 μg/ml). Binding was visualized using a goat anti-mouse biotinylated antibody for 1 h, followed by streptavidin-peroxidase complex which was detected with diaminobenzidine and hydrogen peroxide. Controls were performed using an isotype-matched irrelevant antibody at the same dilution. No counter-staining was used since this would mask nuclear staining. The slides were mounted with Depex and staining evaluated by conventional light microscopy.

Protein analysis by Western blotting

In order to estimate the relative amount of each form of PR, Western blotting was used. The frozen tissue was homogenized by Polytron (Ultra-Turrax T25 from Janke and Kunkel, Staufen, Germany) in 5 ml of lysis buffer (TEG: 10 mM Tris–HCl pH 7.4, 1 mM EDTA

and 10 % glycerol) in presence of an antiproteolytic mixture containing aprotinin 77 mg/ml, leupeptin 1 mg/l, pepstatin A 1 μg/l, bacitracin 100 μg/ml, PMSF 40 μg/l.

The homogenate was clarified by centrifugation at 10 000 *g* for 20 min and the protein concentration determined (DC-Protein Assay, Bio Rad, Hemel Hempstead, Herts, UK). 40 μg of protein extracted from leiomyomata and adjacent myometrium were separated on 7.5% SDS–polyacrylamide mini-gels then transferred electrophoretically at 200 mA for 1 h using a semi dry transfer method (LKB Bromma Multiphor II) to a PVDF membrane (Immobilon, Millipore), using an LKB continuous buffer without methanol (glycine 39 mM, Tris 48 mM, sodium dodecyl sulphate 0.0375%). The conditions used for separation and transfer were chosen after pilot experiments which showed that shorter transfer times significantly altered the ratio of PR-A:PR-B in favour of PR-A (the lower molecular weight form). Increasing the transfer times from those described above did not alter the observed ratio indicating that transfer was complete. Efficient transfer of both forms is essential for accurate estimation of the ratio. After blocking for 1 h at 37°C with PBS containing 10% low fat milk powder (Marvel®) and 0.1% Tween, the membrane was incubated overnight with the monoclonal anti-PR antibody (Novocastra) at a dilution 1/100. The membrane was then washed five times with PBS containing 0.1% Tween, incubated for 1 h at room temperature with horseradish peroxidase-linked sheep anti-mouse IgG at a dilution 1/10 000 and after further washing the immunoreactive proteins were revealed using the ECL reagents and quantified by densitometry (Computing Densitometer Model 300A, Molecular Dynamics, Kent, UK).

An extract of T47D breast carcinoma cells was used as positive control; PR-A and PR-B have been reported to be equimolar in these cells. Cells were harvested at 75% confluence from a 75 cm^2 flask by direct lysis in 500 μl of sample buffer and 3 μl was loaded onto the separating gel.

Preparation of total RNA and RNase protection assay (RPA)

Total RNA was isolated from ~0.5 g frozen tissue pieces using the guanidinium isothiocyanate method (Chomczynski and Sacchi, 1987). A probe for use in the RPA was designed to include a region where the two transcripts differ. The fragment was generated by PCR (position +732 to +911), on the total PR cDNA (kindly provided by P.Kastner), the major transcription sites being described as (+1, +15 for PR-B, +751, +761, +842 for PR-A) in the sequence described by Kastner *et al.* (1990a). The primers used were: 5′-AGTCCAGTCGTCATGACT-3′ and 5′-CATACCTATCTCCCTG-GAC-3′. The amplified DNA was then cloned using PCR Script SK(+) and sequenced to confirm its identity. The in-vitro transcription of the cloned fragment produced a probe of 192 nucleotides (179 + 13 of plasmid). Hybridization of this to PR-B mRNA gives a predicted protected fragment of 179 nucleotides (nt). Hybridization with PR-A mRNA gives several protected fragments between 161 and 151 nt due to multiple initiation sites (Kastner *et al*., 1990a). The transcription/labelling reaction was carried out using 60 μCi [^{32}P]UTP (Amersham International) and the Maxiscript T7 polymerase kit from Ambion (Austin, TX, USA). To isolate the probe, it was run on a denaturing 4% acrylamide gel and the band containing full length probe was excised. The probe was eluted from the gel in 350 μl of elution buffer by incubation overnight at 37°C. A riboprobe for GAPDH was used as an internal standard against which to compare PR-A and PR-B levels. As GAPDH mRNA is more abundant than PR mRNA in all cells the GAPDH probe was synthesized at 10-fold lower specific activity. This ensured that the GAPDH probe could be used in excess and a similar exposure time could be used for both probes. The century RNA marker from Ambion was used as the molecular weight marker.

The labelled PR-A/PR-B and GAPDH probes (4×10^4 cpm) were added to 30 μg of each sample RNA. Following hybridization and RNase digestion the protected fragments were loaded onto a 6% denaturing polyacrylamide sequencing gel. The gel was fixed, dried and autoradiographed at −70°C with intensifying screens. Following exposure the film was developed and densitometry was performed on the resultant PR-A, PR-B and GAPDH bands. mRNA from T47D cells and yeast were our positive and negative controls repectively.

Statistics

The paired Wilcoxon rank sum test was applied to the values for evaluation of differences between PR concentrations in the leiomyomata and the myometrium. The Wilcoxon rank sum test for unpaired samples was used to compare PR amounts in the same tissue type for the different phases of the cycle.

Results

Immunohistochemistry

Using the antibody which recognizes both forms of the PR, nuclear staining was seen, with a greater number of stained nuclei present in leiomyomata compared to myometrium. Furthermore the staining intensity within individual cells was stronger in fibroid tissue than adjacent myometrium (Figure 1, panel A, C, D). Using the antibody for PR-B (B30), there was again nuclear staining with a greater number of cells stained in leiomyomata than in myometrium (Figure 1, panel B, E, F). In both tissues the staining of PR-B was fainter than that observed with the antibody which recognizes both PR-A and PR-B. However, quantitative comparisons between the two staining patterns cannot be made. No cyclical fluctuation of staining either in myometrium or leiomyomata was detectable for either of the antibodies. The staining with the irrelevant mouse IgG did not show any staining (data not shown).

Western blotting for PR-A and PR-B

To obtain a more quantitative assessment of the levels of immunoreactive PR in the leiomyomata and myometrium and to estimate accurately the ratio of the different forms of receptor, Western blotting was used. The monoclonal antibody NCL-PGR was used since this antibody recognizes a common epitope on PR-A and PR-B.

Figure 2 shows that immunoreactive bands of the expected sizes for PR-A (94 kDa) and PR-B (120 kDa) were readily detectable in the fibroid and myometrium of all patients tested; however, there was great variation in amounts of PR between individuals. In the majority of cases (8/11) there was more PR in the fibroid tissue than the myometrium (Figure 3C). In both fibroid and myometrium (11/11 and 10/11 respectively) there was more PR-A than PR-B (1.6-fold) (Figure 3B, A). Both of these differences were highly significant using the Wilcoxon test on paired samples ($P < 0.01$). However, there was no apparent change in PR amounts related to the phase of the menstrual cycle.

PR-A and PR-B mRNA concentration

To determine whether the increased amounts of PR in leiomyomata observed by immunocytochemistry and Western blotting

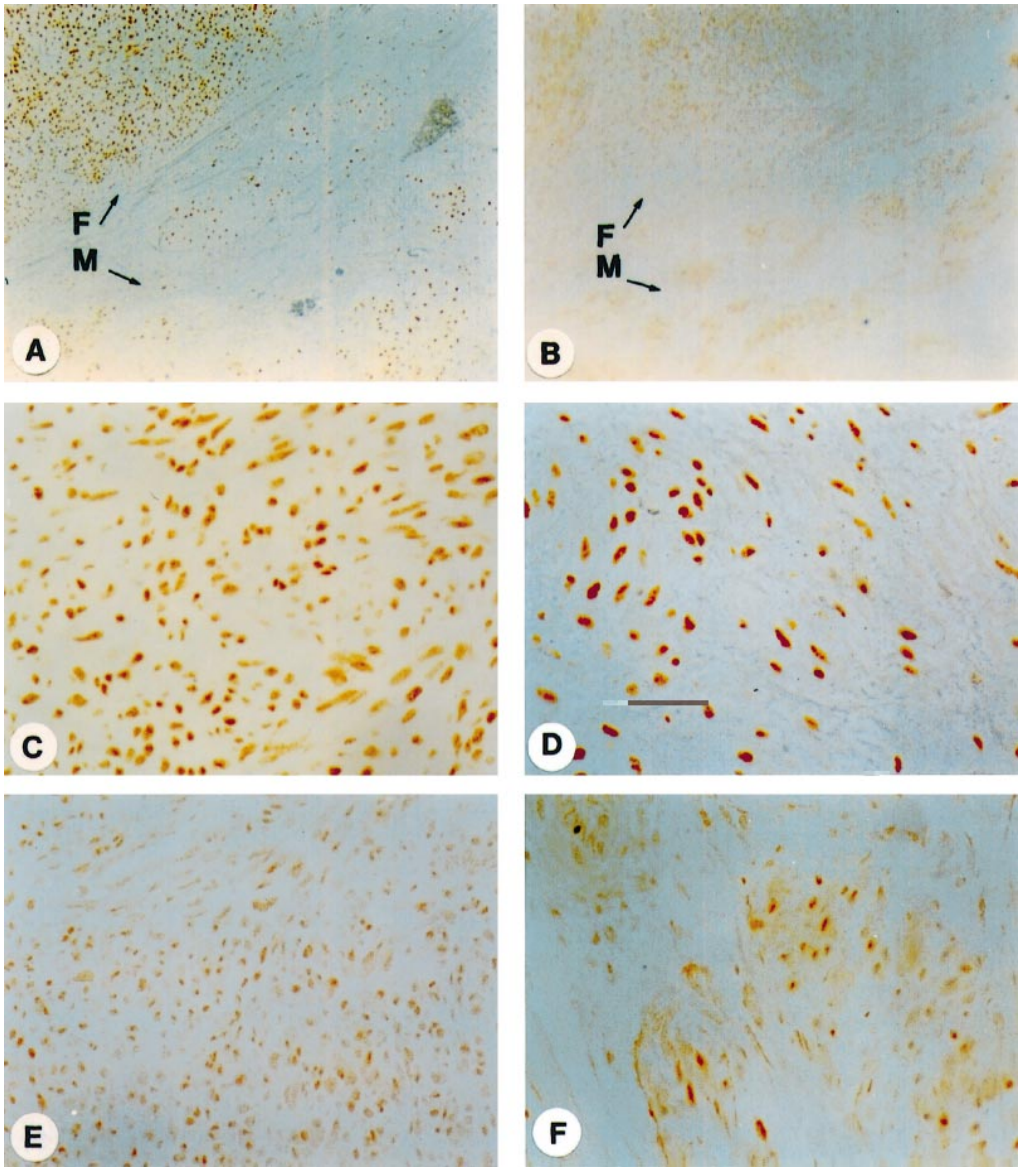

Figure 1. Immunohistochemical staining of progesterone receptor (PR) on cross-section leiomyomata/myometrium. The illustrations are late secretory phase, but the staining has been studied throughout the cycle (data not shown). M indicates myometrium and F fibroid. **A**, cross-section leiomyomata/myometrium (×100) stained with an antibody recognizing PR-A and PR-B. **B**, same cross-section (×100) stained with a PR-B specific antibody. **C**, leiomyomata (×400) stained with an antibody recognizing PR-A and PR-B. **D**, adjacent myometrium (×400) stained with an antibody recognizing PR-A and PR-B. **E,** leiomyomata (×400) stained with a PR-B specific antibody. **F**, adjacent myometrium (×400) stained with an antibody recognizing PR-A and PR-B.

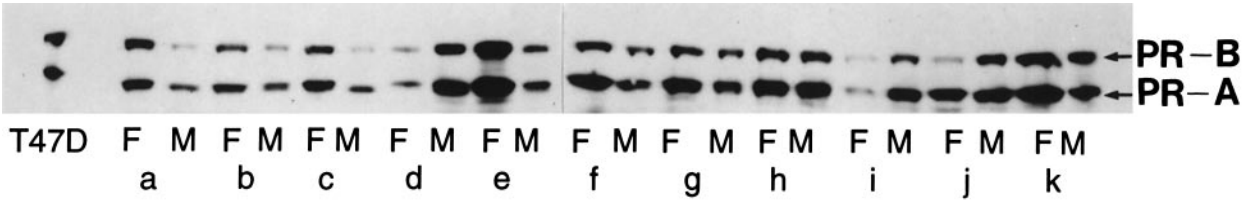

Figure 2. Western blot of 40 µg total protein from leiomyomata and corresponding myometrium, in pairs, from 11 untreated women. (a–e) paired samples in the proliferative phase; (f–k) paired samples in the secretory phase.

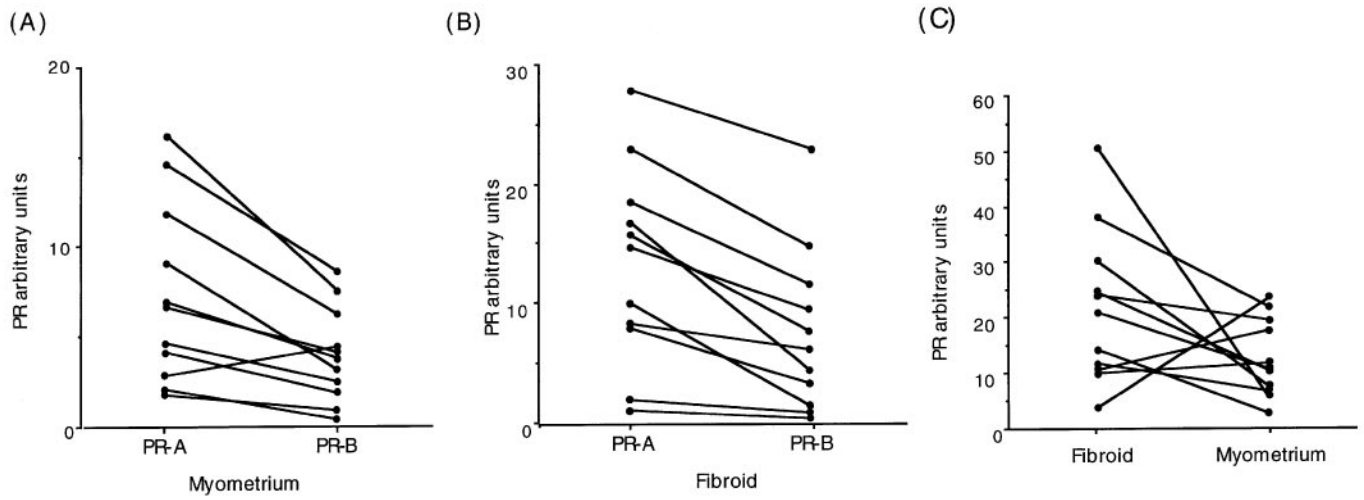

Figure 3. Graphical representation of the Western blot results, expressed in arbitrary optical density units. (**A**), comparison of PR-A and PR-B in myometrium. (**B**), comparison of PR-A and PR-B in leiomyomata. (**C**), total progesterone receptor (PR) in leiomyomata versus total PR in myometrium.

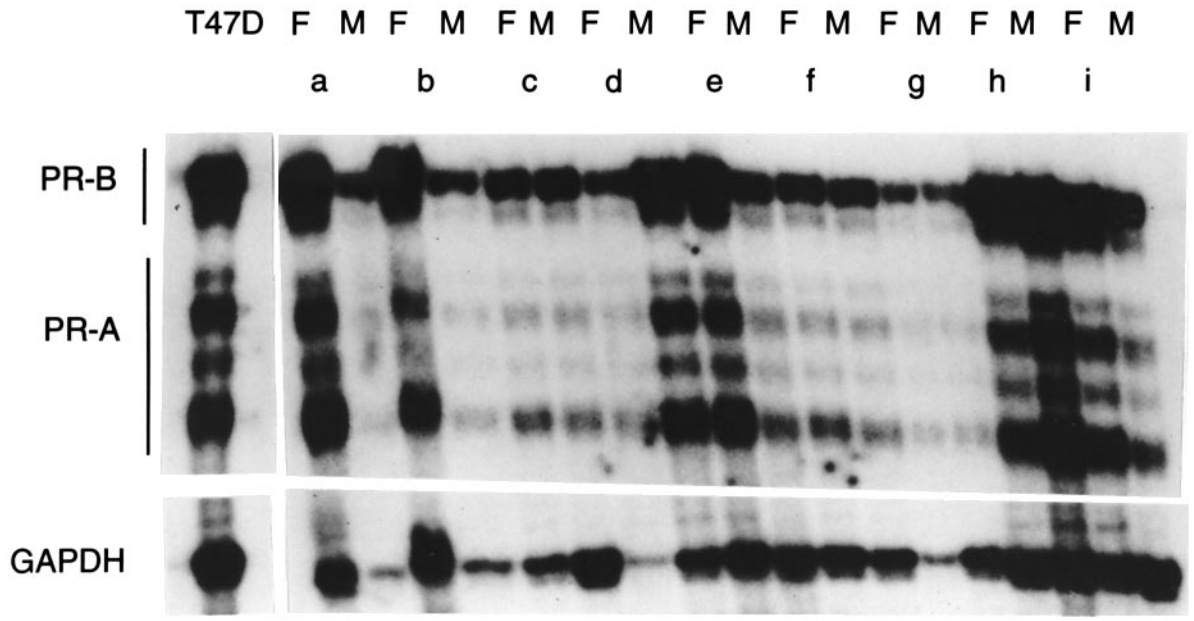

Figure 4. Part of gel containing RNAse protection assay of 30 μg total cellular RNA from leiomyomata and adjacent myometrium in pairs. Transcripts encoding progesterone receptor (PR)-A PR-B and GAPDH are marked on the left. PR-B mRNA appears as a doublet of approximately 179 nucleotides, PR-A mRNA appears as four bands between 161 and 151 nucleotides.

were paralleled by changes in the steady state concentrations of the mRNA encoding PR-A and PR-B we carried out RNase protection assays. Since the probe used spans the region where the mRNA differs it allowed the amounts of PR-A and PR-B mRNA to be determined independently in the same RNA sample. Previous work has relied on Northern blotting which cannot distinguish the different PR transcripts.

The RPA showed four bands for PR-A as there are several initiation sites [as described (Kastner *et al*., 1990a) and confirmed by the primer extension, data not shown]. The doublet labelled PR-B in Figure 4 corresponds to the mRNA that have the potential to encode PR-B since they include the ATG at positions 744–746 (numbering according to Kastner *et al*., 1990a). The optical densities for all the bands corresponding to the PR-A and PR-B transcripts were summed and reported per unit of GAPDH. We concluded that there was no difference between leiomyomata and myometrium in the mRNA expression for either of the forms (Figure 5A, B). There was no significant fluctuation throughout the cycle in either of the tissues.

Discussion

The interactions of progestins, antiprogestins and the two forms of the PR are complex and understanding this is a prerequisite to rational clinical treatment. To investigate the possible role of progesterone in the pathogenesis of leiomyomata we have determined the ratio of immunoreactive PR-A

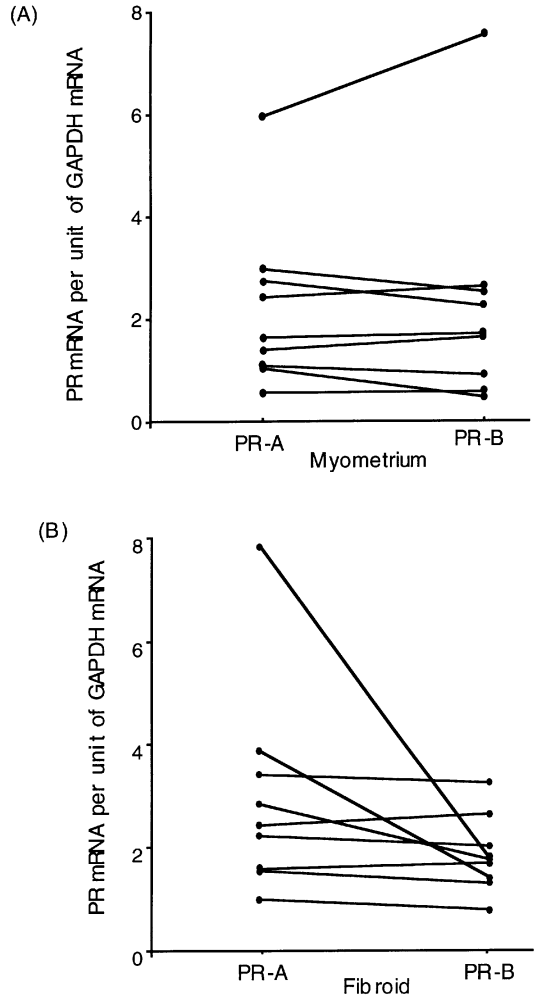

Figure 5. Graphical representation of the RNase protection assay results showing the ratio of progesterone receptor (PR)-A and PR-B mRNA to GAPDH mRNA in both myometrium and leiomyomata.

and PR-B and also the mRNA encoding these proteins in both myometrium and leiomyomata.

Our results show that there is a significant increase in total PR in leiomyomata compared to normal myometrium. This was determined by immunocytochemistry and Western blotting. This supports the view that progesterone plays a role in fibroid growth. One hypothesis to explain this would be that leiomyomata escape completely from oestrogen regulation. This is supported by the observation that PR is elevated in breast carcinoma cells cultured in the absence of oestrogen but in medium containing a high concentration of insulin (Katzenellenbogen and Norman, 1990), and the description of the absence of PR in breast carcinoma tissues in presence of oestrogen (Reiner and Katzenellenbogen, 1986; Rio *et al.*, 1987; Savouret *et al.*, 1991).

An alternative hypothesis would be that the leiomyomata are hypersensitive to oestrogen, and become independent of ovarian oestrogen production which would result in a permanent high concentration of PR. The leiomyomata hormonal milieu could also be hyperoestrogenic. For example the presence of specific receptors for oestradiol and metabolism of oestrogen has been demonstrated in leiomyomata (Reddy and Rose, 1979). Similarly Otubu *et al.* (1982) found that the concentration of estradiol was higher in leiomyomata than in normal myometrium and that the conversion of oestradiol to oestrone in leiomyomata was significantly lower when compared to myometrium.

All these hypotheses would predict absence of cyclical variation in fibroid PR, which we have observed here, and this is in agreement with previous reports (Chrapusta *et al.*, 1990; Vollenhoven *et al.*, 1994). This is also consistent with several immunohistochemical studies which described myometrial PR staining with a possible increase of total PR in late proliferative phase but no decline in the late secretory phase (Lessey *et al.*, 1988) or no variation at all (Savouret *et al.*, 1991). The intensity of PR staining in myometrium has also been described as being similar in menopausal women with or without hormone replacement therapy (Amso *et al.*, 1994). A third theory to account for the higher amounts of PR would be that for a given circulating oestrogen concentration, its activity is enhanced by a high number of oestrogen receptors (ER), and the high content of PR is a consequence of overexpression of functional ER, as suggested by Brandon *et al.* (1995).

The concentration of PR-A was found by Western blot analysis to be higher than that of PR-B in both leiomyomata and myometrium. Thus the possible agonist-like effects that antiprogestins can exert via PR-B would be blocked by the dominant negative effect of excess PR-A (Meyer *et al.*, 1990). This gives scientific support to the medical treatment of uterine leiomyomata with RU 486 since this acts as an antagonist when bound to PR-A. Also the dominance of PR-A annuls the agonist-like effects of antagonist-occupied receptor B. Thus the effects of this agent will be those of antagonist.

The consistent observation that PR amounts are higher in leiomyomata than in adjacent myometrium (for example, this study and Vollenhoven *et al.*, 1994) contrasts with the similar amounts of mRNA encoding PR measured in these two tissues (Figure 5). Also we found there was no difference in the amounts of PR-A and PR-B mRNA. Thus there was no relationship between abundance of the PR-mRNA and immunoreactive protein content. The absence of any significant difference between PR-mRNA in leiomyomata and myometrium has been previously reported (Vollenhoven *et al.*, 1994) and these authors also detected an elevation in progesterone binding in leiomyomata. Our data suggest that PR activity can be regulated post-transcriptionally (and possibly post-translationally) and this is in addition to the differential transcription from the two promoters.

Although the mechanism which determines PR levels remains to be established, and there was some apparent variation in the intensity of nuclear staining in both tissues, the high receptor content of leiomyomata seems likely to be crucial for the growth of fibroid. However we have not been able to detect concordance between the cells positive for PR and the proliferative marker Ki-67 by double immunofluoresence (data not shown) in either leiomyomata or myometrium. This could be explained if the action of progesterone was mediated by local growth factors through paracrine or autocrine action. This is supported by the observation that shrinkage of uterine fibroid with GnRH agonists is associated with a

decrease in EGF binding sites (Lumsden *et al.*, 1988). Insulin-like growth factors I and II have also been implicated since the mRNA encoding these factors have been shown to be elevated in leiomyomata compared to myometrium (Giudice *et al.*, 1993).

The growth of leiomyomata is clearly steroid-dependent and there is now strong evidence in favour of the role of progesterone as a growth promoter. The interaction between the ovarian steroids, their receptors and polypeptide growth factors is clearly complex. The data presented here show that while the amount of PR is significantly elevated in leiomyomata compared to myometrium, the ratio of PR-A:PR-B remains unchanged with PR-A dominating. This has relevance for the possible treatment of this condition with antiprogestins. However this appears not to be regulated at the level of steady state mRNA concentration since the amount of the two mRNA is the same and there is no difference between the leiomyomata and the myometrium.

Acknowledgements

We would like to thank Dr Philippe Kastner for the PR cDNA, Dr K.Horwitz for providing the anti PR-B antibody and Dr Ashley King for histological dating of the tissue samples. We would like to thank the consultant, theatre and histopathology staff of Addenbrookes Hospital, Cambridge, for their help in performing this study. We would also like to thank Dr S. Viville for his advice. B.V. was supported by a grant from Schering Health Care, D.S.C-J. by BBSRC Fellowship PDF/22 and A.M.S. by MRC grant G9403371.

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Received on October 4, 1996; accepted on January 30, 1997