Immunoreactive and estrogen-binding estrogen receptors, and progestin receptor levels in uterine leiomyomata and their parental myometrium

S. CHRAPUSTA (*) - B. KONOPKA(*) - Z. PASZKO (*)
W. SIEINSKI (**) - J. SZAMBORSKI (**)

Summary: Estrogen receptor (ER) and progestin receptor (PR) levels in the myometria and uterine leiomyomata of forty-four women were studied. A radiolligand method and an immunoenzymatic method were used for ER measurement, and only a radio-ligand method was used for PR measurement. The leiomyomata contained significantly more PR and estrogen-binding ER than their parental myometria but not the immunoreactive ER per mg of DNA. Nuclear extracts from the myometria contained a high amount of the estrogen-nonbinding immunoreactive ER; in the leiomyomata, the bulk of this particular ER fraction was extracted with cytosol. Dissimilar distribution patterns of immunoreactive, estrogen-nonbinding ER in leiomyomata and normal myometria suggest that an impaired metabolism of ER may contribute to myoma growth.

Key words: estrogen receptors; progestin teceptors; uterine leiomyoma.

INTRODUCTION

Development and growth of uterine leiomyomata during the fertile period of a woman's life point to a link with ovarian function ([1]1. Steroid hormones act through the specific nuclear receptors in target cells ([2, 3]). The receptor level may influence the sensitivity of the cells ([4, 5]) to a given hormone, and the cause for myoma formation and growth was sought in an impaired estrogen receptor (ER) and/or progestin receptor (PR) regulation ([6, 7, 8]). However, the reports on the relationship between ER and PR levels in uterine leiomyomata and their parental myometrium are conflicting.

The aim of our work was to study the relationship between ER and PR levels in uterine leiomyomata and normal myometrium. Until recently, the only method for steroid receptor assay was the one based on specific ligand binding and prone to many interferences. We supposed that the newly available enzyme immunoassay for ER might be helpful in clarifying the existing discrepancies.

(*) Endocrinology Laboratory, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw (Poland)

(**) Department of Pathomorphology, Institute of Obstetrics and Gynecology, Medical Academy, Warsaw (Poland)

METHOD AND MATERIALS

Forty-four uteri from women undergoing hysterectomy for various reasons were included in the study. The patients were aged 32-67 (46±8, mean ±SD) years. Twenty-six of them were regularly menstruating, one was pregnant, one had secondary amenorrhea, and the remaining ones were perimenopausal or postmenopausal. Two myomata from each of 20 uteri and one myoma from each of the remaining ones were taken, and a sample of myometrium adjacent to the leiomyoma(ta) was taken from each uterus. Each sample was divided into 2 parts. One was frozen and stored in liquid nitrogen for receptor studies; the other was fixed in formalin and used for routine histologic study. Only the myometria with normal histological pattern and the corresponding leiomyomata with less than 2 mitoses per 10 high power fields, showing no atypia or necrosis, were used for receptor studies. Both the myometrium and the leiomyoma(ta) had to be available to include a given uterus in the study.

Enzyme immunoassay for ER was done with the Abbott ER1EIA Monoclonal Diagnostic Kit, following the manufacturer's instructions. The methods for cytosol and nuclear extract (0.48 M KCl) preparation were as described previously [9], except the homogenization buffer was that recommended by the manufacturers of the ER-EIA Kit. For the "cytosolic" ER (ERc) and PR (PRc) assay, the cytosols were diluted with homogenization buffer (to 2 mg protein/ml) or glycerol (10% v/v,), respectively. Nuclear extracts were used undiluted. The experimental details for radioligand receptor assays were as described earlier [9]. Briefly, triplicate 100 µl aliquots of cytosol were incubated overnight at 0 °C with 10 µM tritiated estradiol (^{3}H-E$_2$) in presence and in absence of 1 µM nonlabelled diethylstilbestrol (DES), or with 20 µM ^{3}H-promegestone and 1 µM nonlabelled cortisol in presence and in absence of 2 µM nonlabelled promegestone (total volume 200 µl), for ERc or PRc determination, respectively. The details for "nuclear" PR (PRn) assay were the same as for PRc except that 200 µl aliquots of the nuclear extract were used (tot vol. 203 µl). For the "nuclear" ER (ERn) determination, triplicate 200 µl aliquots of the nuclear extract were incubated at 30 °C for 3 hours with 10 µM ^{3}H-E$_2$ in presence and in absence of 1 µM DES (tot. vol. 203 µl). After incubation, the samples were placed on ice, 20 µl of dextran-coated charcoal suspension (DCC, 0.5% dextran, 5% charcoal in homogenization buffer) was added to each sample and the samples were incubated at 0 °C for an additional 10 min with shaking. After pelleting the DCC suspension by centrifugation at 4600×g for 10 min, the radioactivity of 150 µl samples of the supernatant was measured. The receptor binding was found by subtracting the radioactivity of the samples incubated with nonlabelled DES or promegestone (nonspecific binding) from the radioactivity of the samples incubated without the nonlabelled ligands (total binding).

In the majority of earlier studies, the ERc or PRc levels in meiomyomata were expressed per mg of cytosol protein; the same method was used initially in this study. However, the cytosol protein/DNA ratio in leiomyomata was shown to be lower than that in the myometrium [10]. The same was found in our material but only in normally menstruating women (Tab. 1). Therefore in further studies we measured both cytosol protein and DNA levels of the tissues, and the ERc and PRc levels were expressed both per mg of cytosol protein and per mg of DNA. ERn and PRn levels were expressed per mg DNA in nuclear pellet. Protein and DNA were determined by the method of Lowry [11] and the modified method of Burton [12], respectively. Student's "t"-test for paired data was used to test for inter-tissue differences in protein/DNA ratio as well as in receptor levels, and Spearman's rank correlation test was used to test for inter-receptor relationships.

Table 1. – *Cytosol protein to tissue DNA ratio (mean ±SD) in leiomyomas and normal myometroum of the same uteri. Number of samples is given in parentheses; p = significance level of difference.*

	Normally menstruating women		Other patients	All patients
Leiomyoma	17.6±13.7 (26)		22.7±14.2 (14)	19.4±13.9 (40)
	p = 0.001		p = 0.001	
Myometrium	29.5±11.5 (18)	p = 0.05	19.1±5.8 (8)	26.3±11.1 (26)

RESULTS

A very good correlation between the ER-EIA and ER-RLA levels in cytosols from normal myometria as well as in nuclear extracts from leiomyomata was found, but the slopes of the respective regression lines found by means of unweighted least-squares linear regression were significantly higher than 1. Correlation between the ER1RLA and ER-EIA levels in nuclear extracts from normal myometria and in leiomyoma cytosols was poorer; the slopes of the regression lines were not significantly different from 1, but the *y*-intercepts of the lines differed significantly from O (Fig. 1 a b, Tab. 2).

The leiomyomata contained significantly more PRc, ERc-RLA and ERc-EIA per mg of cytosol protein than their parental myometria. The cellular (i.e. per mg of DNA) PRc, ERn-RLA and ERc-RLA but not ERc-EIA levels were significantly higher in the tumours than in the myometria. Mean PRn level was the same in leiomyomata and their parental myometria (Tab. 3).

The ERc-RLA, ERn-RLA and PRc levels in leiomyomata correlated positively with those in normal myometria, whereas no significant correlation was found between the ERc-EIA or ERn-EIA levels in these tissues. PRc level correlated with ERn but not with ERc level in leiomyomata, whereas it correlated with ERc but not with ERn level in normal myometria (Tab. 4).

DISCUSSION

Reports on the relation between steroid receptors in human uterine myomata and normal myometrium are conflicting. Some Authors have found increased ER ([9, 13, 14, 15]) and PR ([9, 10, 14, 15]) levels in leiomyomata; lack of difference ([8, 13, 16, 17]) or decreased leiomyomal ER ([7]) and PR ([18, 19]) levels have also been reported. There are a number of reasons for this inconsistency. Histologic heterogeneity of uterine leiomyomata ([14]), ethnic factors ([15]), hormonal environment ([16, 17, 18, 19]), and lack of standardized methods for receptor extraction and measurement may all play a ro-

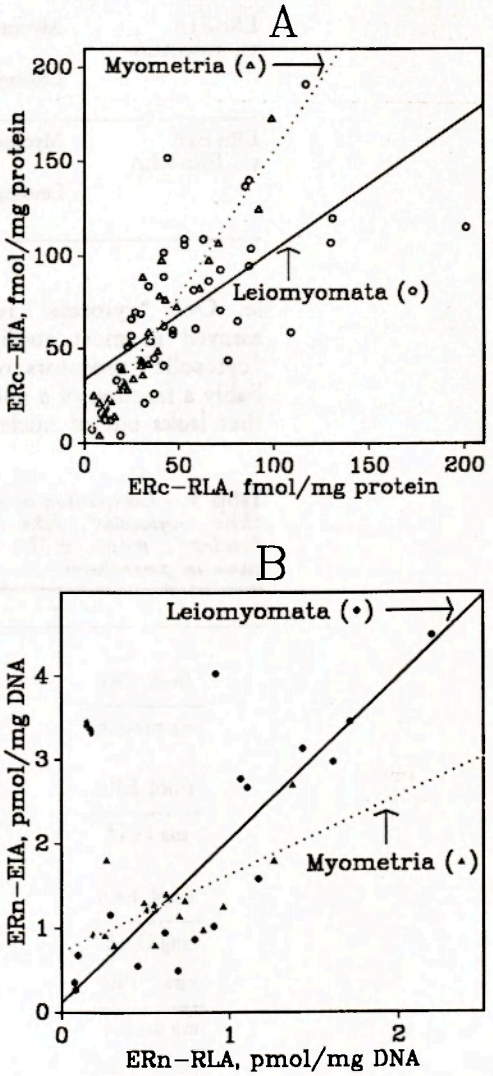

Fig. 1 *a, b.* — Comparison of immunoreactive (EIA) vs. estrogenbinding (RLA) estrogen receptor levels in (*a*) cytosols and (*b*) nuclear extracts from uterine leiomyomata and their parental myometria. Arrows show linear correlation lines.

Table 2. – *Summary of immunoreactive (EIA) vs. estrogen-binding (RLA) estrogen receptor levels comparison. Significance level (p) of correlation coefficients, and 95% confidence intervals are given in parentheses; N = number of samples, ERc = "cytosolic" estrogen receptor, ERn = "nuclear" estrogen receptor.*

Data analyzed	Tissue	N	Linear correlation coefficient	Slope	y-Intercept
ERc-EIA vs. ERc-RLA	Myometrium	34	0.940 (p = 0.001)	1.53 (1.33 - 1.73)	2.5 ([−5.3] - 10.3)
	Leiomyoma	44	0.656 (p = 0.001)	0.70 (0.45 - 1.20)	33.4 (16.5 - 50.2)
ERn-EIA vs. ERn-RLA	Myometrium	15	0.657 (p = 0.01)	0.92 (0.29 - 1.55)	723 (263 - 1183)
	Leiomyoma	18	0.850 (p = 0.001)	1.93 (1.30 - 2.57)	118 ([−553]) - 789)

le. Only "cytosolic" receptor levels were assayed in most studies. However, the "cytosolic" receptors represent most probably a fraction of a nuclear receptors pool that leaks out of nuclei during tissue homogenization due to a low affinity to nuclear components [2]. As shown by our results and others [6, 9, 14], a substantial fraction of ER remains unsolubilized after cytosol extraction. Therefore, the ER le-

Table 3. – *Comparison of estrogen-binding (RLA) and immunoreactive (EIA) estrogen receptor levels (ERc - cytosolic", ERn - "nuclear"), and progestin receptor levels (PRc - "cytosolic", PRn - "nuclear"; means ± SD) in leiomyomas and their parental myometria. Number of samples is given in parentheses.*

		Myometrium		Leiomyoma	
fmol ERc / mg protein	RLA	37±29	(44)	61±43	(64)
		p = 0.001			
	EIA	51±38	(34)	72±42	(44)
		p = 0.002			
fmol ERc / mg DNA	RLA	740±354	(26)	985±779	(40)
		p = 0.03			
	EIA	1039±495	(16)	1077±915	(20)
fmol ERn / mg DNA	RLA	605±291	(25)	949±621	(38)
		p = 0.001			
	EIA	1316±493	(15)	1817±1363	(18)
pmol PRc / mg protein		0.63±0.39	(44)	1.19±0.72	(64)
		p = 0.001			
pmol PRc / mg DNA		15.16±8.05	(26)	20.75±13.32	(40)
		p = 0.001			
pmol PRn / mg DNA		2.89±1.27	(13)	2.76±1.7	(10)

Table 4. – *Correlation between immunoreactive (EIA) and estrogen-binding (RLA) estrogen receptor levels ("cytosolic" - ERc, "nuclear" - ERn) and "cytosolic" progestin receptor (PRc) levels in uterine leiomyomata and their parental myometria. Number of samples is given in parentheses; NS - no significance ($p>0.05$).*

Data analyzed			ER assay	Rank correlation coefficient	Significance level
Myometrial vs. Leiomyomal	ERc		RLA	0.432 (40)	0.01
	ERc		EIA	0.111 (20)	NS
	ERn		RLA	0.463 (38)	0.01
	ERn		EIA	0.259 (18)	NS
	PRc			0.611 (40)	0.001
Myometrial	PRc vs.	ERc	RLA	0.356 (44)	0.02
	PRc vs.	ERc	EIA	0.408 (34)	0.02
	PRc vs.	ERn	RLA	0.338 (25)	NS
	PRc vs.	ERn	EIA	0.314 (15)	NS
Leiomyomal	PRc vs.	ERc	RLA	0.109 (64)	NS
	PRc vs.	ERc	EIA	2.285 (44)	NS
	PRc vs.	ERn	RLA	0.484 (38)	0.01
	PRc vs.	ERn	EIA	0.643 (18)	0.01

Note. The inter-tissue correpations were estimated using receptor levels per mg of DNA; the inter-receptor correlations were estimated using receptor levels per mg of cytosol protein (ERc vs. PRc) or per mg of DNA (ERn vs. PRc).

vel cannot be estimated correctly on the ground of ERc measurement alone.

Most Authors expressed the steroid receptor levels per unit of cytosol protein, and pointed to an increased (ER) or decreased (PR) receptor level as to the reason for growth of leiomyomata [6, 8, 18]. However, the cytosol protein/DNA ratio is lower in the tumours than in the myometrium in menstruating women; therefore the cellular receptor levels in these tissues should be compared in relation to DNA content. In our patients, leiomyomata contained more estrogen-binding (RLA) ERc and ERn per mg of DNA than their parental myometria, but no significant difference was found between the immunoreactive (EIA) ERc or ERn levels in these tissues. In the case of ERn, this apparent discrepancy may result from the smaller number of EIA data, as the ratio of average ERn levels in the tissues was nearly the same for both methods. Nevertheless, the difference between the myometrial and myometrial ER levels is not great and it seems not to account for growth of the tumours.

However, the analysis of RLA vs EIA data suggests that the methods are not always equivalent. The slopes of the regression lines for ER measurements in cytosols from normal myometria and in nuclear extracts from leiomyomata significantly exceeded 1. This results most probably from losing the estrogen-binding ability by a fraction of ER during tissue processing or RLA.

On the other hand, the y-intercept values of the regression lines form ER assays in leiomyoma cytosols and in nuclear extracts from normal myometria were significantly higher than 0. This points to the existence of an immunoreactive, estrogen-nonbinding ER fraction which is not proportional to ER titer and therefore appears not to be a methodological artifact. The origin and biological role of this particular ER fraction may only be speculated on at the moment. In the leiomyomata, this fraction was extracted with cy-

tosol, which points to its low affinity to nuclei and lack of biological action. In normal myometria of patients with uterine leiomyomata, the fraction was found in nuclear extracts; this suggests a high affinity of this ER fraction to nuclei. It is well known that ER binds specifically to the DNA sequences controlling expression of estrogen-regulated genes ([3]). Therefore, the estrogen-nonbinding, immunoreactive ER could influence (inhibit?) estrogen action in normal myometria. Further studies are necessary to verify this hypothesis. It cannot be excluded that some tumour-specific factors influence the redistribution of ER in leiomyoma homogenate. In our study, a correlation was found between the PRc and ERc-RLA but not ERn-RLA level in normal myometria, whereas in leiomyomata a correlation was found between the PRc and ERn-RLA level. This discrepancy may also result from different ER redistribution patterns in leiomyomal and myometrial homogenates.

The increased ER and/or PR levels in uterine leiomyomata may result from the ability of the tumours to synthesize estrogens from androgens ([20]). In women, aromatase activity was found to be several times higher in the leiomyomata than in the myometrium ([21]). An increased leiomyomal estrogen level may enhance ER processing leading to a change of estrogen binding parameters, and may induce an increase in ER and/or PR levels. A reason for the increased ER and PR levels in leiomyomata as compared to those in normal myometrium may be also the in vivo ability of the tumours to synthesize prolactin ([22]). Prolactin may enhance ER expression in the uterus ([23]); however, the normal human myometrium does not produce prolactin in vivo ([22]).

In conclusion: hormone-binding ER and PR levels are increased in uterine leiomyoma cells as compared to those in the cells of their parental myometrium. Dissimilar distribution patterns of immunoreactive, estrogen-nonbinding ER in cytosols and nuclear extracts from the leiomyomata and their parental myometria point out that an impaired ER metabolism may contribute to the increased ER and PR levels in uterine leiomyomata as well as to the growth of the tumours.

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Corresponding Author:
Dr. S. CHRAPUSTA
Endocrinology Laboratory,
Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, bud. "B",
02-034 Warsaw, ul. Wawelska 15 (Poland)