

The Activity of Thymidine Phosphorylase in the Uterine Myomas and the Myometrium in Perimenopausal Women

E. Miszczak-Zaborska^a, J. Greger^a, K. Woźniak^a, U. Kowalska-Koprek^b and T. Pajszczyk-Kieszkiewicz^b

^a Department of Biochemistry, Institute of Physiology and Biochemistry, Medical University of Łódź, Lindleya 6, 90–131 Łódź, Poland

^b Institute of Gynaecology and Obstetrics, Medical University of Łódź, Wileńska 37, 94–029 Łódź, Poland

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The activity of thymidine phosphorylase (dThdPase) in the myometrium and uterine myomas has been investigated in perimenopausal women. Differences in the activity of dThdPase have been found depending on the myoma type, menopause stage and the phase of the menstrual cycle in which the surgery was performed. The enzyme in the cytoplasmic soluble fraction obtained at $50\,000 \times g$ was the most active in cellular leiomyomas of the follicular phase, the least in adenomyomas of the luteal phase of the menstrual cycle, whereas its activity in myometrium was always unchanged. Greater differences can be observed in the activity of dThdPase after a partial purification of the enzyme from myomas.

It seems that the increase in dThdPase activity may point to its correlation with transient, premalignant tumor which may later transform into malignant forms.

Introduction

Thymidine phosphorylase (dThdPase: EC 2.4.2.4.) catalyses the reversible phosphorolysis of thymidine, deoxyuridine and their analogues to their respective bases and 2-deoxyribose-1-phosphate (Iltzch *et al.*, 1985). This enzyme activity increases in several types of malignant tumors compared to the normal tissues (Takebayashi *et al.*, 1995; Takebayashi *et al.*, 1996; Luccioni *et al.*, 1994). dThdPase has been reported to be identical to the platelet-derived endothelial cell growth factor: PD-ECGF (Usuki *et al.*, 1994; Moghaddam *et al.*, 1992; Sumizawa *et al.*, 1993; Barton *et al.*, 1992; Furukawa *et al.*, 1992). This growth factor stimulates the growth and chemotaxis of endothelial cells in vitro and possesses angiogenic activity in vivo (Ishikawa *et al.*, 1989). Recombinant PD-ECGF has dThdPase activity (Sumizawa *et al.*, 1993), and dThdPase has angiogenic activity (Haraguchi *et al.*, 1994). Expression of PD-ECGF/dThdPase plays an important role in the promotion of angiogenesis in human breast cancer (Toi

et al., 1995). The angiogenic activity of PD-ECGF/dThdPase contributes to the progression of some tumors with high PD-ECGF/dThdPase activity (Miyadera *et al.*, 1995). However, the exact mechanism by which dThdPase is angiogenic remains to be proven and it is unclear how dThdPase in malignant tissue is involved in cancer proliferation.

In this study we investigated dThdPase activity in various types of uterine myomas – the most common benign tumors in females and in adjacent myometrium tissues removed during both phases of the menstrual cycle in pre- and menopausal status.

Materials and Methods

Materials

35–53 years old women were treated surgically due to:

- leiomyomas: 16 – in the luteal phase of the menstrual cycle; 4 – in the follicular phase of the menstrual cycle; 4 – in the menopause.
- adenomyomas: 4 – in the follicular phase of the menstrual cycle; 4 – in the luteal phase of the menstrual cycle; 4 – in the menopause.
- cellular leiomyomas: 4 – in the follicular phase of the menstrual cycle.

Reprint requests to Dr. Miszczak-Zaborska.
Telefax: 4842-78-24-65.

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The evaluation was made each time by the histopathologists. The patients had not been treated hormonally before surgery.

Enzyme preparation

dThdPase was partially purified according to the method described by Yoshimura for human placenta (Yoshimura *et al.*, 1990) with our own modifications. The tissues were homogenized in 4 volumes of ice cold buffer B [1 mM EDTA 0.02% 2-mercaptoethanol, 2 mM phenylmethanesulfonyl fluoride (PMSF), 10 mM tris(hydroxymethyl)amino-methane-maleate, pH 6.5] and centrifuged at $50000 \times g$ for 1 h. The supernatant was mixed overnight with 40% saturation of ammonium sulfate at 4 °C, then the precipitate was dissolved in buffer B and dialyzed against this buffer. The dialysed solution was applied to a DEAE-Sepharose (Pharmacia) column and eluted with linear gradient of NaCl (50–250 mM). Then the active fractions were pooled and stored at -70 °C, or were taken for analysis.

Enzyme assays

dThdPase activity was assayed by the spectrophotometric method described by Yoshimura *et al.* (1990), using the transformation of thymine from thymidine in the presence of arsenate. The incubation mixture of 0.5 ml final volume contained 0.1 M tris(hydroxymethyl)aminomethane -arsenate buffer (pH 6.5), 10 mM thymidine and crude or partially purified enzyme. After 1 h incubation at 37 °C, the reaction was stopped by adding 0.5 ml 1 N NaOH and the thymine formed was measured with absorbance at 300 nm. The protein content was determined according to the method described by Bradford (1976).

One unit of activity of dThdPase (U) was defined as the amount of the enzyme which was required to form 1 μ mol of thymine per 1 h. Specific activity was defined as the number of the enzyme activity units per milligram of protein.

Statistical analysis

All results are presented as means \pm SD for four myometria and uterine myomas or more. The

Table I. The activity of dThdPase (U/mg protein) in uterine leiomyoma and myometrium in the luteal phase.

Numbers	Age (years)	Cycle (days)	Leiomyoma			Myometrium		
			Crude extract	Fraction after DEAE- Seph.	Purification	Crude extract	Fraction after DEAE- Seph.	Purification
1	30	31	0.18	4.43	24.9			
2	45	29	0.12	4.55	37.4			
3	30	28	0.16	4.92	30.2			
4	44	29	0.16	4.28	27.6	0.18	2.29	12.9
5	35	28	0.16	5.23	32.7			
6	34	27	0.14	3.06	22.4			
7	34	30	0.09	4.86	52.9			
8	44	29	0.04	2.87	64.1	0.12	0.95	8.1
9	42	27	0.07	3.25	49.6			
10	44	28	0.17	4.86	29.5	0.18	1.27	7.1
11	41	28	0.18	4.68	26.0			
12	43	29	0.12	4.94	40.5	0.16	1.99	12.1
13	48	23	0.10	4.12	43.4			
14	42	26	0.13	4.27	32.3			
15	35	28	0.18	4.73	27.0			
16	48	26	0.12	5.65	47.9	0.14	1.24	9.1
X	40	28	0.13	4.42	36.8	0.15	1.55	9.9
\pm	6	2	0.04	0.78	11.5	0.03	0.57	2.5

1 unit of enzyme activity (U) is defined as the quantity that catalyzes the formation of 1.0 μ mol of a free thymine per hour. Specific activity of dThdPase is expressed as U/mg protein. p-values were calculated using Mann-Whitney test. No statistical differences were observed between leiomyoma and myometrium in crude extract ($p > 0.05$). Statistically significant differences were observed between leiomyoma and myometrium in fraction after DEAE-Sepharose ($p < 0.05$).

Mann-Whitney test was used for calculation of statistical significance of differences between groups. The level of significance was set at $p < 0.05$.

Results and Discussion

We studied the activity of dThdPase in uterine leiomyomas and myometrium removed from 16 women in the premenopausal period in the luteal phase of the menstrual cycle (Table I). In the cytoplasmatic, soluble fraction obtained at $50\,000 \times g$ the activity of dThdPase in leiomyomas ranged from 0.04 U/mg protein to 0.18 U/mg protein; and in myometrium from 0.12 U/mg protein to 0.18 U/mg protein (Table I). Greater differentiation of dThdPase activity in leiomyomas than in myometrium was revealed during luteal phase, although mean enzyme activity of 16 leiomyomas was only slightly lower than that of myometrium; the differences were statistically insignificant (0.13 U/mg protein for leiomyomas vs. 0.15 U/mg protein for myometrium; $p > 0.05$) (Table I). Therefore, these most commonly occurring tumors did not exhibit enhanced dThdPase activity as compared to corresponding myometrium. These tumors, although qualified as benign by some histopathologists (Rosai *et al.*, 1996) cause many troubles such as pain or bleeding. Sometimes they can be dangerous due to their great sizes, especially in pregnant women. Some authors think, that leiomyomas or at least some of them may undergo malignant transformations leading to leiomyosarcomas (Rosai *et al.*, 1996; Rummelink *et al.*, 1996).

The higher enzyme activity, however, is found in leiomyomas investigated in the follicular phase

with estrogen as the dominating hormone revealing still greater differentiation of dThdPase activity (0.45 ± 0.17 U/mg protein for the leiomyoma and 0.16 ± 0.02 U/mg protein for the myometrium; $p < 0.05$) than in the luteal phase when progesterone is the predominant hormone (Fig. 1).

A higher dThdPase activity is also found in leiomyomas removed in the initial menopause (0.40 ± 0.05 U/mg protein for the leiomyomas and 0.14 ± 0.02 U/mg protein for the myometrium; $p < 0.05$) with estrogen as the dominating hormone (Fig. 1).

We compared the activity of dThdPase leiomyomas in the cytoplasmatic, soluble fraction obtained at $50\,000 \times g$ with the activity of the enzyme of cellular leiomyomas, adenomyomas and myometrium in this fraction (Fig. 1). The arrangement of dThdPase activity based on the criterion of its decreasing activity is as follows: cellular leiomyomas of the follicular phase of menstrual cycle > leiomyoma of the follicular phase > leiomyoma of the menopausal period > adenomyoma of the follicular cycle phase > adenomyoma of the menopausal period > leiomyoma of the luteal phase > adenomyoma of the luteal phase (Fig. 1). The activity of dThdPase in all studied myomas removed in the luteal phase does not differ statistically significantly from that in myometrium, while in all myomas removed in the follicular phase the dThdPase activity is statistically significantly higher than in the myometrium removed in this phase of the cycle. Taking into consideration the homology between dThdPase and PD-ECGF (Usuki *et al.*, 1994; Moghaddam *et al.*, 1992; Sumi-

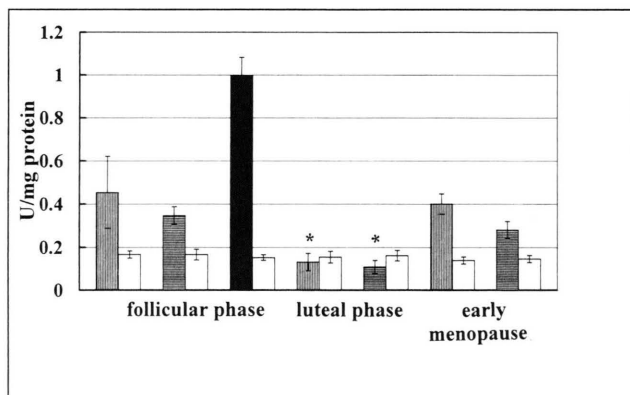


Fig. 1. The activity of dThdPase in the cytoplasmatic soluble fraction obtained at $50\,000 \times g$ in ■ leiomyoma, ▒ adenomyoma, ■ cellular leiomyoma and □ myometrium.

1 unit of enzyme activity (U) is defined as the quantity that catalyzes the formation of $1.0 \mu\text{mol}$ of free thymine per hour. Specific activity of dThdPase is expressed as U/mg protein.

Each value – the mean \pm SD for four experiments except leiomyoma and myometrium luteal phase (\pm SD for sixteen and five experiments, respectively).

* NS – mean non significant (i.e. $p > 0.05$) between leiomyoma and myometrium, adenomyoma and myometrium in luteal phase.

Statistically significant differences were observed between the remaining myomas and myometrium ($p < 0.05$) (The Mann-Whitney test).

zawa *et al.*, 1993) it can be presumed that myomas grow during the follicular phase and early menopause.

The highest dThdPase activity occurs in cellular leiomyomas of the follicular phase of (1.00 ± 0.09 U/mg protein) and the lowest in adenomyoma of the luteal cycle phase (0.11 ± 0.01 U/mg protein).

Some authors suggest, that the pyrimidine nucleoside phosphorylase activity may be a new prognostic factor of tumor malignancy (Takahashi *et al.*, 1995; Moghaddam *et al.*, 1995). Reynolds's results clearly demonstrate that expression of the PD-ECGF is higher in ovarian cancer than in benign tumors (Reynolds *et al.*, 1994). On the other hand, Saeki reports that PD-ECGF/dThdPase may be a useful biological marker of angiogenesis, which in adenomas may prognosticate the formation of colon tumors (Saeki *et al.*, 1996). Therefore, dThdPase expression is closely associated with the promotion of angiogenesis and hepatic metastases in gastric cancer (Maeda *et al.*, 1996). Tumor growth invasion and metastasis all require angiogenesis (Folkman, 1990). Prognosis of individual cancer patients is profoundly influenced by the intensity of these pathophysiological processes. Cellular leiomyomas exhibit increased cellularity and vascularity as compared with leiomyomas and adenomyomas (Rosai *et al.*, 1996). We obtained a 2.2 fold higher dThdPase activity in cellular leiomyomas of the follicular phase as compared with leiomyomas and a 2.9 fold higher dThdPase activity as compared with adenomyomas of the same cycle phase. It could testify to the highest growth rate of cellular leiomyomas or it could indicate that cel-

lular leiomyomas studied by us may be a transient form leading to more malignant uterine tumors.

The activity of dThdPase in leiomyomas of the follicular and luteal phase of the menstrual cycle and early menopause augments about 40 fold after purification of the enzyme by fractionation with 40% saturation of ammonium sulfate and chromatography on DEAE-Sephadex. In the myometrium and the other myomas it does not augment significantly (Table I, Fig. 2). dThdPase purified from leiomyomas is an accessible material for studying biochemical parameters determining its characteristics in benign human tumors. However, because of various degrees of purification of the enzyme after DEAE-Sephadex, depending on the type of the tumor, it seems better, for comparative reasons, to study dThdPase activity in crude extract.

Summing up, the dThdPase activity is the lowest in adenomyomas, higher in leiomyomas and the highest in cellular leiomyomas during follicular phase of the cycle. The dThdPase activity is higher in all types of the studied myomas removed in the follicular phase of the menstrual cycle and in the early menopause than in the myomas removed in the luteal cycle phase. The dThdPase activity in leiomyomas and adenomyomas obtained during luteal phase is similar to the dThdPase activity of the normal myometrium. Thus the dThdPase activity changes according to different benign uterine myoma types and to the phase of the menstrual cycle. The dThdPase activity does not increase in benign uterine tumors in the luteal phase of the menstrual cycle, compared to the normal tissues.

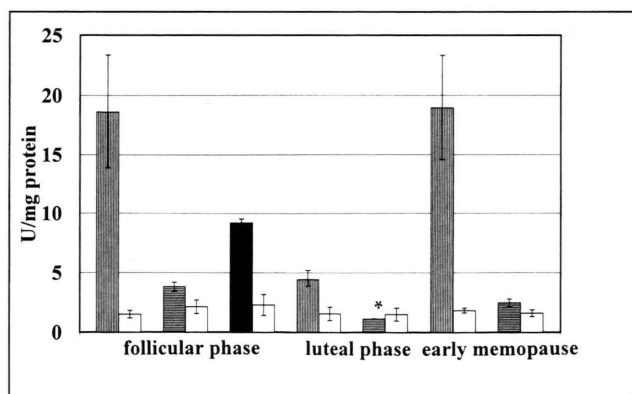


Fig. 2. The activity of dThdPase in a partially purified fraction (fraction after DEAE-Sephadex) in leiomyoma, adenomyoma, cellular leiomyoma and myometrium.

1 unit of enzyme activity (U) is defined as the quantity that catalyzes the formation of $1.0 \mu\text{mol}$ of free thymine per hour. Specific activity of dThdPase is expressed as U/mg protein.

Each value – the mean \pm SD for four experiments except leiomyoma and myometrium luteal phase (\pm SD for sixteen and five experiments respectively).

* NS – mean non significant (i.e. $p > 0.05$) between adenomyoma and myometrium in luteal phase.

Statistically significant differences were observed between the remaining myomas and myometrium ($p < 0.05$) (The Mann-Whitney test).

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