Activity Lysosomal Enzymes in Rat Skin Fibroblasts after Treatment with Progesterone and New Gestagen ABMP

A. B. Liga, T. V. Ukhina, and N. L. Shimanovski

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 145, No. 1, pp. 50-52, January, 2008
Original article submitted July 16, 2007

The effects of a new synthetic gestagen 17α-acetoxy-3β-butanoyloxy-6-methyl-pregna-4,6-dien-20-on (ABMP) and reference drug progesterone on rat skin fibroblasts were evaluated by variations in lysosomal enzyme activity (cathepsin D and β-glucosidase). Our results suggest that ABMP exhibits lysosomotropic properties, which depended on its concentration and time of treatment. The direct effect of progesterone on lysosomal enzyme activity in skin fibroblasts was compared to the influence of systemic treatment with gestagens on skin lysosomes. The data indicate that local application of gestagen preparations holds much promise for the therapy of skin diseases accompanied by increased proliferation (*e.g.* psoriasis).

Key Words: *17α-acetoxy-3β-butanoyloxy-6-methyl-pregna-4,6-dien-20-on; gestagens; progesterone; cathepsin D; β-glucosidase*

Gestagens are extensively used in clinical practice and, particularly, in gynecology for the therapy of dysfunctional metrorrhagia, sterility, amenorrhea, mastopathy, and mastodynia [6].

Previous studies showed that progesterone and some other sex steroids regulate a variety of pathophysiological processes in the skin during psoriasis, aging, and wound healing [7,10]. Progesterone decreases vascular permeability, reduces the severity of cyclic edema in the connective tissue stroma, and inhibits proliferation and mitotic activity of the epithelium in organs and target cells [10]. Progesterone has antiinflammatory activity, which is similar to that of glucocorticoids. These properties of progesterone are mainly related to its influence on the lysosomal apparatus in skin cells [3,5]. Lysosomal enzymes are involved in the development of skin diseases, including atopic dermatitis and psoriasis [2,5]. A detailed study of the mechanism for action of progesterone and other gestagens should be performed to use these drugs in the therapy of skin diseases.

Here we studied the effects of single treatment with a new synthetic gestagen 17α-acetoxy-3β-butanoyloxy-6-methyl-pregna-4,6-dien-20-on (ABMP) and reference drug progesterone on activity of lysosomal enzymes (cathepsin D and β-glucosidase) in fibroblasts of intact rat skin.

MATERIALS AND METHODS

Experiments were performed on cultured fibroblasts. These cells were isolated from the skin of newborn male rats and cultured in Carrel glass vessels in standard DMEM (PanEko) containing 10% fetal bovine serum (PanEko), 100 μg/ml L-glutamine (N. F. Gamaleya Institute of Epidemiology and Microbiology), and 40 μg/ml gentamicin sulfate (Verofarm). For subculturing or experiments the fibroblasts were harvested from the glass surface with 0.25% trypsin. Enzyme activity of trypsin was inactivated with 5-10 ml complete medium (DMEM and serum). The number and viability of cells were estimated in a Goryaev chamber after staining with

Department of Molecular Pharmacology and Radiobiology, Medical and Biological Faculty, Russian State Medical University, Moscow. ***Address for correspondence:*** ariuna.liga@gmail.com. A. B. Liga

0007-4888/08/1451-0044

0.1% trypan blue. The hormone in final concentrations of 10^{-7}, 10^{-5}, and 10^{-3} M was added to the suspension of fibroblasts. Total cathepsin D activity, free cathepsin D activity [1], and β-glucosidase activity [14] were measured. Total enzyme activity was estimated with Triton X-100 in a final concentration of 0.1%. Functional activity and binding of the enzyme to lysosomal membranes were evaluated from the free/total activity ratio (in percents). Protein content was measured by the method of Lowry [12].

The results were analyzed by nonparametric Kruskal—Wallis test and Newman—Keuls test.

RESULTS

Comparison of our results with previous data on rat skin homogenates (derma and epidermis) showed that activity of lysosomal enzymes in fibroblasts is similar to that in the derma of male rats (Table 1) [4].

Progesterone induces a dose-dependent decrease in total activity of cathepsin D and β-glucosidase in fibroblasts, which attests to its possible direct effect on the enzymes. This effect is not mediated by progesterone interaction with the receptor, because normal skin fibroblasts have no progesterone receptors [3,8]. The decrease in the percentage of free enzyme activity was observed only after addition of progesterone in the highest concentration (10^{-3} M). These changes were probably related to the stabilizing effect of progesterone on the lipid bilayer of lysosomal membranes. Progesterone in lower doses did not produce this effect. Similar results were obtained in previous *in vitro* studies of the effect of progesterone on tissue homogenates from intact skin of male rate [3]. Enzyme activity returned to normal 24 h after single treatment with the hormone, which was probably associated with metabolic transformation of progesterone and synthesis of new enzymes.

As differentiated from progesterone, free cathepsin D activity in fibroblasts decreased by 22.2% after 30-min incubation with ABMP in a concentration of 10^{-7} M. Total enzyme activity remained practically unchanged under these conditions. Cathepsin D activity in fibroblasts after 1-h incubation with 10^{-7} M ABMP did not differ from that observed after exposure to this compound for 30 min. The percentage of free enzyme activity decreased by 32.4% under the influence of ABMP in a concentration of 10^{-5} M and remained unchanged over 12 h after treatment. This parameter returned to

TABLE 1. Effect of Gestagens on Activity of Lysosomal Enzymes (μmol/min/mg protein, *M*±*m*)

Preparation	Cathepsin D			β-Glucosidase		
	total activity	free activity	percentage of free activity, %	total activity	free activity	percentage of free activity, %
Control	0.30±0.04	0.18±0.03	57.7±4.0	1.00±0.06	0.38±0.04	39.0±2.3
Progesterone, 30-min incubation						
10^{-7} M	0.20±0.04*	0.10±0.02*	55.1±0.7	0.80±0.04	0.28±0.02*	36.7±1.5
10^{-5} M	0.17±0.01*	0.09±0.02*	55.0±1.5	0.78±0.05*	0.31±0.05	37.0±1.0
10^{-3} M	0.15±0.01*	0.07±0.02*	46.6±1.2*	0.58±0.05*	0.12±0.02*	23.0±1.2*
ABMP, 30-min incubation						
10^{-7} M	0.30±0.02	0.14±0.01*	44.2±1.2	1.05±0.03	0.40±0.04	38.7±1.2
10^{-5} M	0.30±0.04	0.09±0.03*	25.3±2.0*	1.20±0.05*	0.48±0.02*	42.8±0.8
10^{-3} M	0.29±0.01	0.06±0.04*	21.5±1.5*	1.30±0.05*	0.59±0.05*	46.5±1.2*
Progesterone, 1-h incubation						
10^{-7} M	0.21±0.05*	0.11±0.01*	56.3±2.3	0.80±0.07	0.30±0.07	37.8±2.0
10^{-5} M	0.20±0.04*	0.10±0.01*	55.5±3.0	0.80±0.05	0.32±0.06	37.2±2.1
10^{-3} M	0.18±0.04*	0.08±0.01*	46.5±3.0*	0.60±0.95	0.14±0.05*	23.4±3.0*
ABMP, 1-h incubation						
10^{-7} M	0.29±0.03	0.14±0.04*	43.2±4.1*	1.10±0.05	0.36±0.02	38.2±3.3
10^{-5} M	0.30±0.04	0.07±0.02*	22.1±3.5*	1.15±0.01	0.52±0.02*	45.3±3.0*
10^{-3} M	0.25±0.04	0.045±0.030	18.1±3.0*	1.20±0.02	0.60±0.01*	51.0±4.0*

Note. Mean values of 4-6 experiments; *$p<0.05$ compared to the control.

normal in the follow-up period. ABMP in a concentration of 10^{-3} M was more potent that progesterone in decreasing the percent of free enzyme activity (Table 1).

ABMP in a concentration of 10^{-7} M had little effect on β-glucosidase activity. As differentiated from progesterone, ABMP in a concentration of 10^{-5} M increased β-glucosidase activity. Free enzyme activity increased by 25% after 30-min exposure to ABMP in a concentration of 10^{-5} M, which reflected a decrease in β-glucosidase binding to the membrane and are probably associated with activation of the enzyme. After 1-h incubation with ABMP, total β-glucosidase activity in skin fibroblasts did not differ from that after 30-min incubation, while free activity increased by 37%, which was probably related to stimulation of *de novo* enzyme synthesis (Table 1). Previous studies showed that this effect is typical of glucocorticosteroids [4]. It cannot be excluded that ABMP possesses partial glucocorticoid activity.

Hence, progesterone and ABMP produce various effects on lysosomal enzyme activity in rat skin fibroblasts. Progesterone has a modulatory effect on both enzymes. The maximum decrease in enzyme activity and increase in the strength of enzyme binding to the lysosomal membrane are observed under the influence of progesterone in a concentration of 10^{-3} M. As differentiated from progesterone, ABMP increases total and free activities of β-glucosidase. Of particular interest is the fact that gestagen inhibits activity of cathepsin D involved in proteolytic activation and proteolytic degradation of intracellular proteins and, probably, playing a role in invasion and metastatic dissemination of malignant cells due to its destructive effect on the extracellular matrix. This is why cathepsin D often serves as a biological marker of malignant tumors [13]. Cathepsin D expression increases during wound healing, psoriasis, and skin cancer [11]. Moreover, cathepsin D is an important mediator of cell apoptosis (increase in caspase activity and change in the mitochondrial membrane potential are preceded by activation of cathepsin D) [9]. Study of lysosomal enzyme activity during skin inflammation and evaluation of the effect of gestagens are of considerable interest.

Both hormones can be used for the therapy of skin diseases associated with increased activity of cathepsin D (*e.g.*, in psoriasis). It should be emphasized that ABMP increases β-glucosidase activity, which is also elevated in psoriasis. Therefore, progesterone decreasing β-glucosidase activity is preferable for the treatment of this condition. The direct effect of progesterone on lysosomal enzyme activity in skin fibroblasts was compared to the influence of systemic treatment with gestagen on skin lysosomes. The data indicate that local application of gestagen preparations holds much promise for the therapy of skin diseases accompanied by increased proliferation (*e.g.* psoriasis).

REFERENCES

1. A. J. Barrett, R. T. Din, and D. Dingle, *Lysosomes. Methods of Study* [in Russian], Ed. J. Dingle, Moscow (1980).
2. L. F. Kaznacheeva, N. A. Rychkova, N. N. Mayanskaya, *et al*., *Allergologiya*, No. 3, 12-16 (1999).
3. P. V. Sergeev, T. V. Ukhina, G. Zh. Kalmagambetova, and M. M. Shegai, *Byull. Eksp. Biol. Med*., **122**, No. 8, 146-149 (1996).
4. T. V. Ukhina and M. M. Shegai, *Ibid*., **115**, No. 3, 265-267 (1993).
5. M. G. Shubich and T. G. Avdeeva, *Arkh. Patol*., No. 2, 3-8 (1997).
6. B. S. Apgar and G. Greenberg, *Am. Fam. Physician*, **62**, No. 8, 1839-1846, 1849-1850 (2000).
7. S. C. Gilliver, F. Wu, and G. S. Ashcroft, *Thromb. Haemost*., **90**, No. 6, 978-985 (2003).
8. S. Im, E. S. Lee, W. Kim, *et al*., *J. Korean Med. Sci*., **15**, No. 6, 647-654 (2000).
9. K. Kågedal, M. Zhao, I. Svensson, and U. T. Brunk, *Biochem. J*., **359**, Pt. 2, 335-343 (2001).
10. N. Kanda and S. Watanabe, *J. Dermatol. Sci*., **38**, No. 1, 1-7 (2005).
11. G. Leto, F. M. Tumminello, M. Crescimanno, *et al*., *Clin. Exp. Metastasis*, **21**, No. 2, 91-106 (2004).
12. O. H. Lowry, N. J. Rosebrough, A. L. Farr, *et al*., *J. Biol. Chem*., **193**, No. 1, 265-275 (1951).
13. T. Nomura and N. Katunuma, *J. Med. Invest*., **52**, Nos. 1-2, 1-9 (2005).
14. H. L. Patel and H. O. Tappel, *Ibid*., **23**, No. 3, 159-168 (1969).