Anabolic Properties of *Dioscorea Deltoidea Wall* Furostanol Glycosides

V. A. Dubinskaya, L. B. Strelkova, I. S. Vasil'eva, S. S. Nikolaeva, L. B. Rebrov, and V. A. Paseshnichenko

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 126, No. 8, pp. 178-181, August, 1998
Original article submitted October 30, 1997

The natural agent deltostim is a mixture of furanostanol steroid glycosides characterized by a high anabolic and a low androgenic activities. In doses of 5-30 mg/kg it stimulates the production of proteins and nucleic acids in the skeletal and cardiac muscles. These doses do not suppress biopolymer production in the liver, which indicates that the agent is not toxic for animals.

Key Words: *steroid glycosides; anabolic activity; RNA, DNA, globular, and fibrillar protein production*

The use of synthetic anabolics for the treatment of many diseases, for improving adaptation to exercise in athletics, and for body weight gain in animals in agriculture is associated with many untoward effects because besides anabolic these agents possess androgenic activity [5]. Recent studies have been focused on agents of plant origin exerting no direct androgenic effects, stimulating protein production, and activating some enzymes involved in energy metabolism [3]. For example, steroid glycosides are wide-spectrum biostimulators which were isolated from many plants.

Deltostim (DS) isolated from a suspension cell culture of *Dioscorea deltoidea wall*, strain DM-05 (Institute of Plant Physiology, Russian Academy of Sciences) is a mixture of the furostanol glycosides deltoside and protodioscine (2:3). Its toxicity is low and it is soluble in water, which differs it favorably from synthetic steroids. Pharmacological studies of DS revealed immunomodulating activity and ability to stimulate ovulation in mammals [2] and prompted investigation of its anabolic effects.

Research Center for Biological Structures, Institute of Medicinal and Aromatic Plants; A. N. Bach Institute of Biochemistry, Russian Academy of Sciences, Moscow

We studied the anabolic activity of DS and the main mechanisms underlying this activity: protein and nucleic acid production in animal tissues (skeletal and cardiac muscles and liver).

MATERIALS AND METHODS

Experiments were carried out on 3-week-old castrated male rats initially weighing 40-60 g. The animals were castrated under light ether narcosis as described previously [4]. The rats were fed the standard diets. All agents were dissolved in normal saline and injected intramuscularly in a single dose of 0.5 ml for 7 days starting from the day of castration. In the first series of experiments, the anabolic properties of DS were studied. The rats were divided into 6 groups, 5 animals per group: 1) controls injected with normal saline; 2) DS in a total (injected for 7 days) dose of 5 mg/kg; 3) 10 mg/kg DS; 4) 30 mg kg DS; 5) 190 mg/kg DS; and 6) 35 mg/kg testosterone propionate. On the 8th day, 24 h after the last injection, anabolic effect of DS was assessed by changes in body weight. For integral assessment of anabolic properties of the drug, the anabolic index was calculated as the ratio of myotropic activity (increment in the weight of *m. levator ani,* MLA) to androgenic activity (increment in the

0007-4888/98/0008-0800$20.00

TABLE 1. Effect of DS on Body Weight Gain and Anabolic Index of Castrated Male Rats (*M±m*)

Drug, total dose, mg/kg		Body weight gain, g	MLA weight mg	VP weight, mg	Anabolic index: MLA weight/VP weight
Control		6.4±1.9	30.36±6.02	35.30±1.58	0.86±0.12
DS,	5	19.0±3.5	55.62±7.33	44.35±5.65	1.26±0.10
	10	22.4±2.8	59.84±8.90	44.33±6.12	1.32±0.15
	30	13.2±2.9	56.84±13.69	51.49±10.41	1.16±0.21
	190	10.7±2.4	46.25±5.12	45.34±3.81	1.02±0.10
Testosterone propionate, 35		11.2±1.8	39.87±5.11	58.63±8.12	0.68±0.12

weight of ventral prostate, VP). The index value >1 indicates that the studied drug is anabolic, less than 1 that it is androgenic [5].

In the second series, the effect of DS on the production of biopolymers RNA, DNA, and protein in rat skeletal and cardiac muscles and liver was studied. Experiments were similar to the first series, but only two doses were used: 5 and 30 mg/kg.

Protein and nucleic acids were measured in tissue homogenates degreased with chloroform:methanol mixture (2:1). RNA was separated from DNA by hydrolysis in 0.3 N KOH at 37°C for 1 h. Hydrolysate was acidified by $HClO_4$ on the cold to attain the 0.5 N concentration. RNA in supernatant was measured by spectrophotometry as described previously [8]. The precipitate containing DNA was hydrolyzed in 0.5 N $HClO_4$ at 70°C for 20 min. DNA in hydrolysate was measured as described previously [6]. Protein was measured by a modified biuret method [1]. Activation of protein and nucleic acid production in tissues was assessed by the ratios RNA/protein, DNA/protein, and RNA/DNA. The content of protein and nucleic acids was assessed per g wet tissue.

In the third series, the effect of DS on the content of the connective tissue protein collagen was studied in the skeletal and cardiac muscles. Hydroxyproline was measured as described previously [9]. Preliminary hydrolysis of tissues was carried out in sealed ampoules in 6 N HCl at 120°C for 8 h. Possible changes in collagen macromolecules caused by various doses of the drug were assessed by the amount of hydroxyproline released into water during 10-min warming of tissue fragments to 65°C (Verzar's test [10]).

RESULTS

The results presented in Table 1 demonstrate the anabolic effect of DS. All doses of furostanol glycosides markedly increased body weight in comparison with the control group. The highest effect was observed with the least doses: 5 and 10 mg/kg. The body weight gain in this group of animals was almost 3.5 times as high as that in the control. The increase in the weight of MLA, the target organ for anabolics, was greater than the increment in the weight of VP. At a dose of 5 mg/kg, the MLA weight increased by 83.2% during the experiment, while the weight of VP increased by only 25.6%. Similar changes were observed at a dose of 30 mg/kg: the MLA weight increases by 87.2% and that of VP by 45.9%.

The anabolic index was >1 for all doses of DS, indicating that the anabolic effect predominates and the androgenic effect is negligible or null. The synthetic steroid testosterone propionate, a classical drug of comparison, induced an increment in body weight in comparison with the control group, but exerted both anabolic and androgenic effects, causing

TABLE 2. Effect of DS on Production of Protein and Nucleic Acids in Muscle Tissue (*M±m*)

DS dose, mg/kg		Myocardium			Skeletal muscle		
		RNA/protein, $\times 10^3$	DNA/protein, $\times 10^3$	RNA/DNA	RNA/protein, $\times 10^3$	DNA/protein, $\times 10^3$	RNA/DNA
Control		5.4±0.2	25.0±0.2	0.22±0.03	6.7±0.4	11.5±0.1	0.58±0.02
DS,	5	8.1±0.4	11.2±0.1	0.72±0.10	9.8±0.2	12.9±0.1	0.75±0.05
	30	24.0±0.6	16.6±0.3	1.44±0.40	9.5±0.3	13.8±0.2	0.66±0.05

TABLE 3. Effect of DS on Protein and Nucleic Acid Production in Liver Tissue of Rats (M±m)

DS dose, mg/kg	RNA/protein, $\times 10^3$	DNA/protein, $\times 10^3$	RNA/DNA
Control	11.8±0.2	51.8±0.4	0.23±0.02
DS, 5	13.2±0.1	43.6±0.5	0.30±0.03
30	10.9±0.3	46.3±0.4	0.22±0.02

testosterone propionate (Table 4). The proportion of soluble collagen released with the low-molecular-weight fraction into hot water decreased. Anabolic and androgenic drugs may accelerate collagen maturation in the skeletal muscle by enhancing its cross-linking.

The content of hydroxyproline in the myocardium doubled after 30 mg/kg DS. Heart weight virtually did not change. An increase in the proportion of collagen in the myocardium was observed after the synthetic steroid retabolil, which is widely used in cardiology. Due to the ability to stimulate the production of collagen in the myocardium, DS can be used in cardiology for accelerating reparative processes in the heart during the treatment of myocardial infarction.

TABLE 4. Effect of DS on Hydroxyproline Content in the Skeletal and Cardiac Muscles (*M±m*)

Drug dose, mg/kg	Hydroxyproline content in skeletal muscle, g/100 g dry tissue		Hydroxyproline content in myocardium, g/100 g dry tissue	
	total	after heating at 65°C	total	after heating at 65°C
Control	0.084±0.018	0.038±0.013	0.055±0.03	0.045±0.004
DS, 5	0.071±0.013	0.048±0.016	0.051±0.012	0.043±0.008
30	0.088±0.027	0.047±0.018	0.115±0.026	0.076±0.013
Testosterone propionate, 35	0.071±0.23	0.068±0.010	0.045±0.012	0.043±0.007

a notable increase in the weight of VP. Its anabolic index was much lower than 1.

Then we evaluated the effect of DS on the production of RNA, DNA, and proteins in the skeletal and cardiac muscles. The drug injected in a dose of 30 mg/kg stimulated the myocardium (Table 2).

After injection of DS, the ratios of biopolymers in the myocardium changed: the RNA/protein ratio also increased more than 4-fold and the RNA/DNA ratio increased, indicating active protein production in the muscle. Injection of the drug in a dose of 5 mg/kg changed the DNA/protein and RNA/DNA ratio, but to a lesser extent than the dose of 30 mg/kg. The effect of DS on the skeletal muscle in the studied doses was lower than its effect on the myocardium, which is probably caused by hypodynamia during the experiment: the anabolic effect is the highest in intensive exercise.

Study of DS effect on the liver revealed no appreciable stimulation of protein and nucleic acid production (Table 3). The content of protein and RNA in the liver slightly increased after a dose of 5 mg/kg. It is important that the studied doses did not suppress the biopolymer production in the liver, which indicates that DS is not toxic for animals.

Changes in the content of the connective tissue component of the skeletal and cardiac muscle induced by DS were assessed by the content of hydroxyproline.

The total content of hydroxyproline in the skeletal muscle virtually did not change after DS and

Thus, studies of the biologic effects of DS on organs and tissues of experimental animals demonstrated its low toxicity and high anabolic effect, consisting in stimulation of protein and nucleic acid production.

REFERENCES

1. J. Bailey, in: *Methods of Protein Chemistry* [in Russian], Moscow (1965), pp. 77-80.
2. I. S. Vasil'eva and V. A. Paseshnichenko, *Priklad. Biokhim.*, **31**, No. 2, 238-242 (1995).
3. P. K. Kintya, *Zh. Vsesoyz. Khim. Obshc. D. I. Mendeleeva*, **33**, No. 5, 584-585 (1988).
4. Ya. D. Kirshenblad, in: *Practical Course of Endocrinology* [in Russian], Moscow (1989), pp. 121-122.
5. V. A. Rogozkin, *Anabolic Androgenic Steroid Metabolism* [in Russian], Leningrad (1988).
6. A. S. Spirin, *Biokhim.*, **23**, 656-662 (1958).
7. E. Baker, S. Landgrehe, R. Mathur, *et al.*, *Fertil. Steril.*, **38**, 38-41 (1982).
8. A. Fleck and H. N. Munro, *Biochim. Biophys. Acta*, **38**, 571-583 (1962).
9. H. Stegemann, *Z. Phys. Chem.* (Leipzig), **33**, 41-43 (1958).
10. F. Verzar, *Schweiz. Med. Wochenschr.*, **93**, 1036-1038 (1963).