4. M. D. Mashkovskii, in: *Drugs* [in Russian], Moscow (1993), Vol. 1, p. 682.
5. P. V. Sergeev and E. N. Mineeva, *Vestn. Akad. Med. Nauk SSSR*, № 6, 57-61 (1990).
6. P. V. Sergeev and N. L. Shimanovskii, *Receptors* [in Russian], Moscow (1987).
7. J. Tepperman and H. Tepperman, in: *Metabolic and Endocrine Physiology: An Introductory Text, Yearbook Medical Publ.*, Chicago (1987).
8. I. V. Torgov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, № 2, 299-317 (1982).
9. C. A. Dujovne and W. S. Harris, *Ann. Rev. Pharmacol. Toxicol.*, **29**, 265-288 (1989).
10. E. R. Ferguson and B. S. Katzenellenbogen, *Endocrinology*, **100**, № 5, 1242-1251 (1977).
11. B. S. Katzenellenbogen, E. R. Ferguson, and N. C. Lan, *Ibid.*, **100**, № 6, 1252-1259 (1977).
12. O. H. Lowry *et al.*, *Analyt. Biochem.*, **83**, № 2, 346-356 (1977).

Correlation of Antiinflammatory and Hormonal Activity of Derivatives of the 8,16-Diazasteroid Series

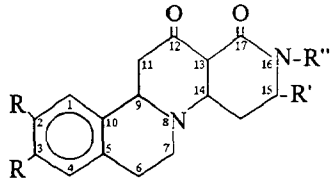
B. B. Kuz'mitskii, B. A. Volynets, N. A. Mizulo, V. M. Nasek, and O. F. Lakhvich

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 119, № 2, pp. 168-171, February, 1995
Original article submitted March 9, 1994

Antiinflammatory activity and mechanism of action are studied for seven compounds of the 8,16-diazasteroid series. It is established that the antiinflammatory activity of the compounds is increased on the whole due to the reduced ketofunction in the 12 position of 8,16-diazasteroid as well as for the introduction of methoxy groups in the 2 and 3 position or phenol substitute in the 16 position. The activity of compounds VI and VII also depends on the inflammation model or on the pain reaction and differs significantly from the effectiveness of diclofenac sodium and prednisolone. Unlike the latter, the compounds under study are virtually devoid of hormonal activity.

Key Words: *8,16-diazasteroid; diclofenac sodium; prednisolone; antiinflammatory activity; hormonal effects; structure-activity correlation*

The heterosteroid compounds are of interest as a useful tool for distinguishing antiinflammatory, antiallergic, and immunomodulating effects from hormonal activity [1]. A more profound modification of the structure of 8-azasteroids by the substitution carbon for nitrogen in the 16 position can either boost or weaken the pharmacological effects and makes it possible to perform a purposeful synthesis of compounds with preassigned properties. The aim of the present investigation was to identify the compounds with pronounced antiinflammatory properties among the known 8,16-diazasteroids [2] and to study the mechanisms of their biological effects. The general structure of the compounds under study was as follows:



where I: R=H, R'=R"=H; II: R=H, R'=R"=H, at C_{12}-H; III: R=H, R'=CH_3, R"=H; IV: R=H,

Department of Biological Assays, Institute of Bioorganic Chemistry, Belarus Academy of Sciences, Minsk. (Presented by P. V. Sergeev, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0002-0159$12.50

TABLE 1. Comparative Antiinflammatory Activity of Compounds VI, VII, and Standard Preparations (Percentage of Effect in Relation to Control, $M \pm m$, $n = 7$)

Compound	Antiexudative activity at peak of edema				Permeability of skin vessels	Antiproliferative effect	Antialterative effect	Analgetic effect (acetylcholine seizures)	Antipyretic effect (drop of body temperature in yeast fever, °C)
	thermal	carrageenan	bentonite	histamine					
VI	79.0±1.6***	31.8±5.1**	75.0±1.8***	59.1±2.7***	143.3±13.3***	41.0±3.9**	73.4±7.9***	46.0±7.1**	0.6±0.2**
VII	45.0±7.4**	18.2±2.9*	53.5±11.5***	33.8±8.4*	56.7±13.3***	34.3±5.6**	18.7±12.3	28.1±5.4	1.1±0.1**
Prednisolone	50.0±9.2**	48.4±10.2**	20.1±12.3	–	−60.0±9.8	64.7±10.7**	19.4±11.1	–	–
Diclofenac sodium	27.7±10.1	78.9±1.8***	52.5±10.2***	–	–	–	39.1±9.8*	93.1±5.6***	1.7±0.2**

Note. *$p<0.05$; **$p<0.01$; ***$p<0.001$ in comparison to the control.

R'=H, R"=C_6H_5; V: R_2=H, R_3=CH_3O, R'=R"==H; VI: R=CH_3O, R'=R"=H; VII: R=CH_3O, R'=H, R"=C_6H_5.

MATERIALS AND METHODS

Noninbred Wistar rats (180-200 g) and CC57W mice (18-21 g) of both sexes were used for the study. Antiexudative activity was determined by the effect on rat hind limb edema induced with carrageenan (Pacific Institute of Bioorganic Chemistry, Russian Academy of Sciences), agar (Difco), bentonite (Spofa), and transmitter-type substances (histamine, serotonin, and bradykinin - Reanal) or with thermal burn. The magnitude of edema was estimated by measuring the dorsoplantar diameter of the paw with slide caliper, the results being expressed in percent of the baseline. The percentage of edema inhibition was calculated as well. In view of the edema dynamics, the observations were performed every 15-30 min or every hour during 4-6 h.

The influence on vascular permeability was photometrically evaluated as the content of dye transported from the blood to the skin [3].

Antiproliferative activity was judged from the capacity of the compound to inhibit the formation of experimental granuloma. The substances were injected once a day for 6 days.

The effect of compounds on the alterative processes was assessed from their capability to limit necrotic changes in the inflammatory focus induced by serotonin and urea and was estimated under conditions of a single intramuscular injection of the drugs 1 h prior to the simulation of necrosis.

The analgetic activity was evaluated from the influence on the threshold of pain sensitivity in noninbred mice of both sexes for pain stimulation induced by acetic acid, acetylcholine, and the tail flick and hot plate tests. The percentage of inhibition of convulsions or of the pain threshold was calculated based on sound and motor reactions.

The antipyretic activity was judged from the ability of the compounds to lower the body temperature in hyperthermia induced by baker's yeast injected subcutaneously, or by sodium arachidonate or prostaglandin E_2 (PGE_2) administered in a lateral ventricle of the brain.

The activity of the studied compounds was compared with the known activity of the pharmacopoeial diclofenac sodium (voltaren) and prednisolone (Gedeon Richter).

The compounds with marked inflammatory activity were tested for hormonal activity [4]. Adrenalectomized male CC57W mice were used for the tests. An increase of the glycogen content in the liver and glucose in the blood was evidence of glucocorticoid activity, while the mineralocorticoid action of the compounds was estimated from the alteration of the content of sodium and potassium ions in erythrocytes and blood plasma. The lympholytic effect was assessed by a decrease of the thymus and spleen weight. The influence on the weight of the liver and cardiac and femoral muscle was examined as well.

The tests were performed during the second half of the day in the fall-winter season. The unified methods recommended by the Pharmacologic Committee for experimental study of nonsteroid antiinflammatory drugs were used in the tests. The data were statistically analyzed using the Student t test [5]. For some experimental series ED_{50} was calculated [6].

RESULTS

Enaminoketolactone I unsubstituted in the A and D rings exerts a weak antiedemic activity on the

model of thermal inflammation. The boosting antiexudative effect of compounds III and IV, which contain 15-methyl and 16-phenyl substitute, respectively, manifests itself in a 40-45% inhibition of limb edema mainly at the resolution stage. The presence of a 3-methoxy group in the structure of V does not result in an enhanced antiexudative activity of the compound, which just exhibits a tendency to inhibit thermal edema. Under the action of 2,3-dimethoxy-substituted 8-aza-D-homogonan VI, the attenuation of limb edema at the peak of inflammation attained 79%. This substance is not inferior in activity to prednisolone and diclofenac sodium. Its 15-methylated analog VII is less active than standard preparations. Of interest is diazasteroid II unsubstituted in rings A and D with the absence of a carbonyl group in the 12 position; it is no less active than the 2,3-dimethoxy-substituted derivative of VI in test for antiexudative activity on the model of thermal inflammation.

Reduction of the 12-oxo group results in high antiexudative activity of II, manifested on the model of carrageenan inflammation as a 60-75% ($p<0.01$) weakening of limb edema. The effectiveness of this compound is similar to that of diclofenac sodium. A weak modulation of carrageenan edema was noted for 2,3-dimethoxy-substituted D-homogonan VI. The antiexudative activity was lowered in the 15-methyl analog VII in comparison to compound VI. Both these compounds exhibit an antiexudative effect in both intact and adrenalectomized rats in the test of carrageenan inflammation.

Compound II blocks agar-induced limb edema by 60% whereas substance VII has a low potency and acts only at the resolution stage of inflammation. The antiinflammatory action of compound VI is pronounced. The results of screening showed that in the series studied only compounds VI and VII produced a marked antiinflammatory effect, the pharmacologic spectrum of which is given in Table 1.

Substances VI and VII, like diclofenac sodium, hamper the development of bentonite-induced inflammation when used for therapeutic but not preventive purposes (Table 1).

The testing showed that substance VI has a marked antihistamine effect in the model of paw edema, whereas its 15-methyl analog VII under the same conditions is weak. Compound VII hastens the resorption of bradykinin edema. Neither of these substances is able to inhibit serotonin edema.

Compound VI is 2.5-fold more effective than compound VII for inhibiting capillary permeability. However, the latter is 1.4 times more effective than butadion in normalizing vascular permeability.

Both of these substances possess a weak antiproliferative activity. In regard to inhibiting proliferation in granular tissue, compound VI is 1.5 times less active than prednisolone.

No effect on the alterative changes in the inflammatory focus was noted when testing substances II and VII. However, 2,3-dimethoxy-substituted ketolactone VI limits the necrotic processes in the injury focus by 73%.

All substances studied are capable of raising the threshold of pain sensitivity in mice for intraperitoneal administration of acetic acid and acetylcholine. The ability to delay convulsions caused by acetic acid increases from 12-deoxo analog II to 2,3-dimethoxy analog VII, which has an analgetic activity equal to diclofenac sodium. However, in relation to acetylcholine-induced convulsions, an effect was found only in compounds II and VI. In the tail flick test the 15-methylated analog VII exhibits a more powerful and prolonged analgetic activity, whereas in the hot plate test substance VI is 1.5 times stronger than the 2,3-dimethoxy analog VII.

Compounds VI and VII lower the rectal temperature by 0.6-1°C for yeast-induced fever in rats, and therefore their antipyretic effect is inferior to that of diclofenac sodium. Diazasteroid VI, unlike the 15-methylated analog VII, lowers the body temperature due to the central action of arachidonic acid. Neither of these compounds affects hyperthermia resulting from the central action of PGE_2, but they do decrease PGE_2 formation in brain homogenates.

Compounds I, V, and VI in a single injection fail to alter the glycogen content in the liver and the blood sugar level, attesting to the absence of glucocorticoid activity. The same substances do not exert any substantial influence on the K^+ and Na^+ content in erythrocytes and blood plasma of adrenalectomized mice. Administration of the test compounds does not change the weight of the thymus, liver, and cardiac and skeletal muscle, while the weight of the spleen drops somewhat. On the other hand, substance I reduces the thymus weight by 11% in adrenalectomized rats.

The screening revealed substances with marked antiinflammatory activity among the derivatives of the 8,16-diazagonan series. An increase of such activity is achieved by the introduction of methoxy groups in the 2-3 position or a phenyl substitute in the 16 position. Reduction of 12-ketofunction in the structure of 8,16-diazasteroid unsubstituted

in the phenol ring enhances the antiinflammatory activity. Elimination of a strong polarized carbonyl group boosts the lipoidotropy of a compound, intensifying its antiinflammatory activity. The presence of a 15-methyl group in the 2,3-dimethoxy-substituted diazasteroid causes the efficacy to decline, according to a number of tests. Hormonal activity is not inherent to 8,16-diazagonans VI and VII, which impinge upon many elements of the inflammatory process, whereas unsubstituted diazasteroid I is found to boost glycogen deposition in rat liver. The results of this investigation hold promise of a fruitful search for antiinflammatory substances in the 8,16-diazasteroid series that do not have any perceptible hormonal activity and that differ both from corticosteroids and from nonsteroid antiinflammatory agents.

REFERENCES

1. A. A. Akhrem, F. A. Lakhvich, L. G. Lis, et al., *Izv. Akad. Nauk BSSR. Ser. Khim. Nauk.*, № 6, 81-90 (1982).
2. V. N. Pshenichnyi, O. F. Lakhvich, and V. A. Khripach, *Ibid.*, № 5, 70-74 (1991).
3. M. I. Bushma, P. I. Lukienko, and Yu. M. Ostrovskii, *Farmakol. Toksikol.*, № 4, 481-483 (1977).
4. V. V. Gatsura, In: *Methods of Primary Pharmacological Screening of Biologically Active Substances* [in Russian], Moscow (1974), pp. 112-117.
5. M. L. Belen'kii, *Basic Quantitative Estimation of Pharmacological Effect* [in Russian], Leningrad (1963).
6. B. M. Shtabskii, M. I. Gzhegotskii, M. R. Gzhegotskii, *et al.*, *Gig. San.*, № 10, 49-51 (1980).

Effect of Cortisol and Pertussis Toxin on the cAMP Concentration in Human Lymphocytes

A. S. Dukhanin, L. I. Stankevich, and M. P. Petrova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 119, № 2, pp. 171-173, February, 1995
Original article submitted February 21, 1994

It is demonstrated that pertussis toxin and hydrocortisone potentiate the adenosine-induced rise of the cAMP concentration in lymphocytes. Hydrocortisone elicits an immediate (for the simultaneous addition of adenosine and cortisol) and reversible effect. The effect of pertussis toxin has a latency and is irreversible. Added together, these agents exert no cumulative effect. It is assumed that hydrocortisone and pertussis toxin have the same target - the inhibiting regulatory protein G_i.

Key Words: *cortisol; pertussis toxin; adenosine; cAMP; lymphocytes*

Molecular aspects of corticoid-dependent modulations of the second messenger levels in competent cells have hardly been studied. It has been assumed that glucocorticoid hormones potentiate the effects of adenylate cyclase activators (β-adrenoagonists, adenosine, and prostaglandin E_2) by increasing the coupling of receptor and catalytic subunits of the adenylate cyclase system [5,6].

Department of Molecular Pharmacology and Radiobiology, Russian State Medical University, Moscow. (Presented by P. V. Sergeev, Member of the Russian Academy of Medical Sciences)

Peripheral blood lymphocytes are a convenient model, since their biological response to glucocorticoids has been studied in sufficient detail and is easy to reproduce. They have two types of plasma membrane receptors for adenosine: A_1 and A_2 [2,3]. The interaction between A_2 receptors and adenosine increases the activity of adenylate cyclase and elevates the intracellular cAMP content. The A_1 receptors are coupled to the enzyme via the regulatory G_i protein, which mediates the inhibitory effects of various hormones and neurotransmitters on adenylate cyclase activity [8]. Therefore,

0007-4888/95/0002-0162$12.50