Antiinflammatory Effect of Russian-Made Budesonide in Experimental Noninfectious Pulmonary Granulomatosis

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 131, No. 5, pp. 557-559, May, 2001
Original article submitted January 25, 2001

We studied the antiinflammatory effect of a Russian-made glucocorticoid budesonide administered by inhalation and intraperitoneal injection to rats with experimental noninfectious pulmonary granulomatosis. The antiinflammatory effects of budesonide at both administration routes were similar. Intraperitoneal injection, in contrast to inhalation, produced a systemic effect manifested in suppression of hyperplasia of non-encapsulated lymphoid formations in the mucosa.

Key Words: *experimental noninfectious granulomatosis; budesonide; Wistar rats*

Glucocorticoids are widely used in clinical pulmonology for the treatment of sarcoidosis, idiopathic fibrosing alveolitis, and asthma. Long-term oral therapy with glucocorticoids is associated with numerous side effects, such as steroid ulcers, osteoporosis, diabetes, myopathies, *etc.* The use of aerosol forms helped to reduce the incidence and severity of side effects. Clinical efficiency of aerosol glucocorticoids, high local antiinflammatory activity, and the absence of systemic effect (or minor side effects) were demonstrated [3-5].

We evaluated the antiinflammatory activity of a Russian-made glucocorticoid drug budesonide on the model of noninfectious pulmonary granulomatosis induced by Sephadex A-25.

MATERIALS AND METHODS

Experiments were carried out on 40 male Wistar rats (200-240 g). Two experimental groups (10 animals each) were exposed to Sephadex A-25 inhalations as described previously [2]. Control group consisted of intact rats. Experimental animals daily inhaled or were injected (7 days) with budesonide in a single dose of 1 mg/kg. Budesonide was synthesized at the Laboratory of Chemistry of Natural Compounds, Center for Safety of Bioactive Substances, by Drs. N. N. Gireeva and A. P. Krylov and registered at the Ministry of Health of the Russian Federation as Benacort (registration Nos. 95/212/1, 95/212/5). Budesonide inhalations were carried out under ether narcosis using a powder inhalator (Cyclochaler analog for small laboratory animals) designed at the Institute of Medical Engineering by V. A. Kaznacheev and A. V. Lokhmachev. Bronchoalveolar lavage fluid (BALF) was collected under deep intraperitoneal hexenal narcosis (35 mg/kg) and the absolute number of cells per 1 ml BALF (cytosis) was determined. In smears stained by the Romanowskii—Giemza method, endopulmonal cytogram and absolute cell count were evaluated. For detecting lymphoid follicles, macropreparations of the lungs and small intestine were fixed in 2% acetic acid for 12-18 h. Volume density of lymphoid follicles was evaluated under a ×7 glass using an G. G. Avtandilov grid [1]. Volume density of alveolitis and emphysema were evaluated morphometrically in histological sections stained with hematoxylin and eosin by point counting with the use of Avtandilov grid. The data were processed statistically using Student *t* and Fisher exact tests.

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TABLE 1. Effects of Sephadex A-25 and Budesonide Therapy on the Course of Inflammatory Process in the Lungs of Wistar Rats (*M*±*m*)

Parameter	Control	Sephadex A-25		
		no budesonide	+budesonide	
			inhalations	intraperitoneally
Volume density, %				
alveolitis	—	19.4±2.9	$14.2\pm3.0^{+++}$	$12.3\pm3.1^{+++}$
emphysema	3.5±0.3	$20.1\pm2.3^{***}$	$14.7\pm1.8^{***+++}$	$16.0\pm2.5^{***}$
Absolute counts of cells/ml BALF	120±10	$440\pm50^{*}$	$150\pm20^{+}$	$130\pm30^{++}$
macrophages	104±3	$300\pm7^{*}$	$90\pm12^{+}$	$85\pm13^{+}$
lymphocytes	9±2	$37\pm2^{*}$	35±11	$21\pm7^{+++}$
neutrophils	5±1	$103\pm20^{**}$	$25\pm4^{**++}$	$26\pm11^{+++}$
Volume density of lymphoid follicles in				
bronchial wall	39.2±1.6	$58.8\pm3.4^{***}$	$39.1\pm3.3^{+++}$	$33.3\pm2.0^{+++}$
small intestinal wall	5.0±0.1	$6.6\pm1.5^{**}$	6.4±0.6	5.8±1.3

Note. $^{*}p<0.001$, $^{**}p<0.01$, $^{***}p<0.05$ *vs.* control; $^{+}p<0.001$, $^{++}p<0.01$, $^{+++}p<0.05$ *vs.* effect of Sephadex A-25 without budesonide treatment.

RESULTS

No differences in the general condition between the control and experimental animals were observed during the experiment. On day 7 after inhalation of Sephadex A-25 aerosol, Wistar rats developed granulomatous inflammation with acute bronchitis, alveolitis, and formation of mature macrophage granulomas, paralleled by hyperplasia of non-encapsulated lymphoid formations (bronchial-associated lymphoid tissue). The granulomas were located in the perivascular and peribronchial connective tissue and between alveolar septae. Morphometrical study showed that both aerosol and intraperitoneal administration of budesonide caused a decrease in the volume density of alveolitis and emphysema in comparison with untreated animals (Table 1).

BALF cytosis in budesonide-treated rats decreased 2.9- and 3.4-fold compared to untreated animals (Table 1). This decrease in cytosis in budesonide-treated rats was paralleled by a significant decrease in neutrophil content in BALF both after inhalation and intraperitoneal treatment. However, neither BALF cytosis, nor neutrophil count returned to normal (Table 1). BALF lymphocyte count virtually did not change after inhalation treatment (Table 1). After intraperitoneal injection, the absolute count of lymphocytes significantly decreased compared to untreated rats. Volume density of lymphoid follicles in the bronchial wall decreased after inhalation treatment and, especially, after intraperitoneal injection of budesonide (Table 1).

Morphometric assessment of the volume density of lymphoid follicles in the small intestine showed that treatment with Sephadex A-25 led to hyperplasia of gut-associated lymphoid tissue (Table 1). Budesonide treatment (both inhalations and intraperitoneal injections) led to a decrease in the volume density of the small intestinal lymphoid follicles, but the difference from untreated rats was insignificant.

Hence, pronounced antiinflammatory activity of Russian-made glucocorticoid budesonide was demonstrated in Wistar rats with experimental noninfectious pulmonary granulomatosis. The drug reduced the severity of alveolitis and emphysema and suppressed hyperplasia of lymphoid tissue associated with the bronchi and intestine. Antiinflammatory effect of budesonide did not depend on the administration route, but after intraperitoneal injection the drug exerted a systemic immunotropic effect, manifested by more pronounced suppression of hyperplasia of the bronchial- and gut-associated lymphoid tissue and by decreased BALF cytosis.

REFERENCES

1. G. G. Avtandilov, *Introduction to Quantitative Pathological Morphology* [in Russian], Moscow (1980).
2. O. V. Makarova, V. L. Kovaleva, A. S. Sladkopevtsev, *et al., Pul'monologiya,* No. 1, 76-79 (1996).
3. O. A. Sutochnikova, A. L. Chernyaev, and A. G. Chuchalin, *Ibid.,* No. 4, 78-83 (1995).
4. Kh. Shimbakh and G. Knappe, *Hormone Therapy* [in Russian], Moscow (1988).
5. J. Feldman, D. Funder, and J. Loose, *Steroid Biochem.,* **19,** 141-145 (1987).