Allergic and Immunotoxic Effects of Calcium Ketopantoyl Aminobutyrate

A. L. Karaev, I. D. Treshchalin*, D. A. Bodyagin*, E. R. Pereverzeva*, G. S. Kozlova, and V. I. Gunar

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 139, No. 4, pp. 428-431, April, 2005
Original article submitted January 26, 2004

We studied the allergic and immunotoxic effects of a new promising medicinal preparation calcium ketopantoyl aminobutyrate. Calcium ketopantoyl aminobutyrate produced no negative effects on general mechanisms of humoral and cellular immunity. This preparation did not modulate the anaphylactic reaction to bovine serum, exhibited no mitostatic and lymphotoxic properties, had no effect on the delayed-type hypersensitivity response, and did not produce active cutaneous anaphylactic reaction.

Key Words: *calcium ketopantoyl aminobutyrate; general anaphylactic reaction; active cutaneous anaphylactic reaction; mitostatic and lymphotoxic effects; delayed-type hypersensitivity*

Pantoham (calcium homopantothenate, calcium pantoyl aminobutyrate) was successfully used in clinical practice for more than 20 years. The preparation is used for the correction of nervous system dysfunction in children of different age [1,3]. Sometimes Pantoham is used in the therapy of mental and nervous disorders (adult subjects with residual organic damage to the brain), complications of cerebral stroke, Alzheimer's disease, senile and presenile psychoses, and other diseases (in elderly individuals) [4].

The drawback of Pantoham is its low therapeutic index, which restricts the use of high doses because of the risk of toxic side effects that are particularly undesirable in children. The structure of Pantoham was modified to synthesize a new safe medicinal preparation that would be highly efficient during the therapy of neurological and mental diseases. This preparation received the name calcium ketopantoyl aminobutyrate (KPA-Ca). Pharmacological studies showed that this new compound possesses high neurotropic activity and has several advantages over Pantoham. These data suggest that KPA-Ca is a promising drug. Here we studied possible side effects of this preparation (including allergic and immunotoxic activity).

Laboratory of Pharmacology, State Research Institute of Vitamins; *Laboratory of Pharmacology and Chemotherapy, G. F. Gauze Institute of New Antibiotics, Russian Academy of Medical Sciences, Moscow

MATERIALS AND METHODS

Experiments were performed on guinea pigs (250-300 g) and (CBA×C57Bl)F_1 mice (20±2 g). The animals fed a standard diet and had free access to water.

The doses of KPA-Ca were determined using conversion coefficients for the dose per body surface area. Taking into account the data on acute toxicity of KPA-Ca in mice, the maximum tolerated dose (MTD) of this preparation for guinea pigs is 500 mg/kg.

The general anaphylactic reaction was assessed 2 h and 1 day after administration of a challenge dose of KPA-Ca or bovine serum (BS). Active anaphylactic shock was produced routinely using BS [5]. The animals receiving subcutaneous injection of 0.1 ml physiological saline over 14 days before administration of BS served as the negative control.

Active cutaneous anaphylactic reaction was studied after intraperitoneal injection of 5% aqueous solution of KPA-Ca in a single dose of 50 mg/kg. The animals presensitized with intraperitoneal injection of

0007-4888/05/1394-0434

TABLE 1. Effect of KPA-Ca on Active Cutaneous Anaphylactic Reaction

Group	Sensitization	Challenge	Size of spot, mm^2
1 (KPA-Ca)	50 mg/kg 5% solution subcutaneously	0.1 ml 5% KPA-Ca intracutaneously	7.4±3.5
2 (control)	0.1 ml physiological saline subcutaneously	0.1 ml 5% KPA-Ca intracutaneously	6.8±2.6
3 (control, BS)	0.1 ml subcutaneously	0.1 ml BS intracutaneously	41.3±13.7*

Note. *$p \leq 0.05$ compared to group 2.

0.1 ml BS served as the positive control. KPA-Ca in the challenge dose (0.1 ml, 5% solution) was injected intracutaneously at the peak of the immune response (2 weeks after sensitization). Control animals received 0.1 ml BS. Blue Evans (0.5 ml, 1% solution) was injected intracardially immediately after challenge. The animals were killed after 30 min. Skin fragment in the site of injection was separated and the size of the blue spot on the inner surface of the skin was measured (Table 1).

The conjunctival test was performed on intact guinea pigs and animals intraperitoneally sensitized with 5% aqueous solution of KPA-Ca in a dose of 50 mg/kg. The animals received 5% solution of the preparation on day 14 (1 drop in each eye). The reaction was assessed after 15 min and 24 or 48 h.

The graft-versus-host reaction was conducted to study mitostatic and lymphotoxic activity [6]. (CBA×C57Bl)F_1 and CBA mice served as the recipients and donors, respectively (Table 2).

The delayed-type hypersensitivity response was studied on CBA mice intravenously immunized with sheep erythrocytes (2×10^6 cells per mice). The animals received intraperitoneal injections of 5% aqueous solution of KPA-Ca in doses of 150 mg/kg ($^1/_{10}$ MTD) and 1.5 g/kg (MTD). Sheep erythrocytes (1×10^6 cells) were injected into the paw pad on day 5. Physiological saline was administered into the contralateral paw. Sheep erythrocytes in the challenge dose were injected into the paw of intact animals (control group). Intact mice receiving intraperitoneal injections of 5% KPA-Ca in a dose of 300 mg/kg (total MTD) over 5 days before administration of this preparation in the challenge dose served as the positive control. The animals were killed after 1 day. The paws were amputated and weighted. The response index was calculated as follows:

$$\frac{(P_T\text{-}P_C)}{P_C}\times100\%,$$

where P_T and P_C are the weights of treated and control paws, respectively.

The results were analyzed by methods of variational statistics (Student's *t* test, Turbo-dost5 software).

RESULTS

The study of the general anaphylactic reaction showed that 2 of 10 guinea pigs in group 1 died 1 day after treatment (severe reaction, +++). The state of group 5 animals corresponded to normal (all animals were alive). The state of group 2 guinea pigs remained unchanged after administration of the test preparation in the challenge dose (negative reaction).

The effect of KPA-Ca on anaphylactic shock was studied in 2 series. The animals of groups 3 and 4 received 5 intraperitoneal injections of KPA-Ca in a single dose of 100 mg/kg before and after sensitization

TABLE 2. Mitostatic and Lymphotoxic Effects of KPA-Ca

Scheme of treatment, (CBA×C57Bl)F_1 recipients	Number of transplanted lymph node cells (CBA donors)	Mean number of colonies	Index for inactivation of endocolonies, %
Irradiation, 600 rad	—	9.7±1.4	—
Irradiation (600 rad)+5 mg/kg KPA-Ca	—	10.1±1.8	+1.1
Irradiation (600 rad)+50 mg/kg KPA-Ca	—	9.5±1.7	-0.2
Irradiation (600 rad)+lymph node cells	1×10^6	0.92±0.48	-95.2
Irradiation (600 rad)+lymph node cells+500 mg/kg KPA-Ca	1×10^6	0.95±0.21	-97
Irradiation (600 rad)+lymph node cells+50 mg/kg KPA-Ca	1×10^6	1.2±0.37	-87.7

Note. —, no data.

TABLE 3. Effect of KPA-Ca on Delayed-Type Hypersensitivity Response

Group	Weight of paw		Index of response, %	
	right	left	relative to contralateral paw	relative to intact control
1 (control, immunization with 2×10^6 sheep erythrocytes)	241.0±7.3	124.0±7.8	194	138
2 (control, intact, no immunization)	174.0±6.3	128±11	134	
3 (150 mg/kg KPA-Ca intraperitoneally+ immunization with sheep erythrocytes)	329.0±6.7	133.0±9.6	179	137
4 (1.5 g/kg KPA-Ca intraperitoneally+immunization with sheep erythrocytes)	254.0±14.8	137.0±7.6	192	146
5 (control, 300 mg/kg KPA-Ca intraperitoneally, 5 injections over 5 days before challenge)	168.0±8.2	130.0±7.2	129	96

with BS, respectively. Of 10 guinea pigs in each group 3 animals died in group 3 and 2 animals in group 4 (intensive reaction).

KPA-Ca had no anaphylaxis-provoking activity. Administration of this preparation before and after sensitization did not modulate the course of anaphylactic shock to BS. These data suggest that KPA-Ca has no effect on the general mechanisms of humoral immunity.

The study of active cutaneous anaphylactic reaction revealed no negative effects of KPA-Ca on the immune system. The reference area of the spot on the inner surface of the skin was calculated by multiplication of 2 diameters. This area in treated animals differed by 5.6 times from the positive control group, but not from the negative control group (Table 1).

KPA-Ca produced no conjunctival effect in guinea pigs sensitized with the preparation in a dose of 50 mg/kg. The study of the mitostatic and lymphotoxic effects showed that treatment with the preparation in a dose of 5 mg/kg during irradiation does not decrease the number of colonies in the spleen. Increasing the dose of KPA-Ca to 50 mg/kg was not accompanied by inhibition of colony formation. The number of colonies in animals of both groups did not differ from the control. KPA-Ca had no mitostatic activity and did not suppress migration of colony-forming units or formation of endocolonies under conditions of irradiation.

Administration of allogeneic cells from lymph nodes in a dose of 1×10^6 cells suppressed colony formation. These results indicate that we selected the appropriate dose of lymph node cells, which produced graft-versus-host reaction.

KPA-Ca in a dose of 500 mg/kg did not modulate graft-versus-host reaction and number of colonies during transplantation of cells from allogeneic lymph nodes. Increasing the dose of KPA-Ca to 1.5 g/kg was not followed by a decrease in the number of endocolonies. These data show that KPA-Ca exhibits no lymphotoxic activity (Table 2).

KPA-Ca did not modulate the delayed-type hypersensitivity response. This preparation had no effect on study parameters in animals immunized with sheep erythrocytes (Table 3).

Our findings suggest that KPA-Ca does not modulate the major immune stages. Activity of the preparation should be estimated in further studies.

REFERENCES

1. L. A. Ermolina, A. A. Kashnikova, Yu. B. Kovalenko, *et al*., *Pantoham: A Twenty-Year Experience of Use in Psychoneurology* [in Russian], Moscow (1998), pp. 53-57.
2. V. M. Kopelevich, T. D. Marieva, V. I. Gunar, *et al*., *Ibid*,. pp. 7-13.
3. O. I. Maslova and V. I. Shelkovskii, *Ibid*., pp. 50-52.
4. A. P. Muzychenko, *Ibid*., pp. 14-22.
5. R. V. Petrov, R. M. Khaitov, V. M. Man'ko, *et al*., *Methodical Data on Experimental (Pharmacological) and Clinical Studies of Immunomodulatory Activity of Pharmacological Preparations* [in Russian], Moscow (1984).