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Potential Mutagenicity, Embryotoxicity, and Teratogenicity of Calcium Ketopantoyl Aminobutyrate

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We studied mutagenic, embryotoxic, and teratogenic properties of calcium ketopantoyl aminobutyrate, a preparation proposed as a new drug. Long-term oral administration of calcium ketopantoyl aminobutyrate produced no mutagenic, embryotoxic, and teratogenic effects.

Key Words: *calcium ketopantoyl aminobutyrate; chromosomal aberrations; mutagenicity; embryotoxicity; teratogenicity*

Calcium ketopantoyl aminobutyrate (KPA-Ca) was synthesized at the State Research Institute of Vitamins. Pantogam (calcium homopantothenate, calcium pantoyl aminobutyrate) was used in clinical practice for more than 20 years [2]. This substance served as a prototype for KPA-Ca. Pharmacological studies showed that new compound possesses high neurotropic activity and has several advantages over Pantogam. These data indicate that KPA-Ca is a promising medicinal preparation [4].

Here we studied the safety of long-term treatment with KPA-Ca and evaluated possible mutagenic, embryotoxic, and teratogenic properties of this preparation.

MATERIALS AND METHODS

There is no universal method for evaluating the ability of a test compound to induce mutations. This goal can be achieved through combination of methods for studying various experimental objects.

Potential mutagenicity was studied in the Ames Salmonella/microsomes test. We used indicator strains of *Salmonella typhimurium*, which allowed recording the following mutations: frame shift (TA 98) and base-pair substitution (TA 100). The strategy of experiments with bacterial cultures, study of the strain genotype, principles of museum storage, need of special devices, laboratory glassware, reagents, nutritive media, and solutions, methods for isolation of rat liver homogenates, and preparation of the activation mixture were described elsewhere [4]. Liver S_9 fraction was obtained from rats receiving intraperitoneal injection of Sovol in a single dose of 300 mg/kg 5 days before euthanasia [1]. This fraction was used for exogenous metabolic activation. Ethidium bromide (5 μg per dish) was used in variants with or without the system of metabolic activation (SMA, strain TA 98) to estimate activity of the fraction.

KPA-Ca was dissolved in sterile distilled water (0.1, 1.0, 10.0, 100.0, and 1000.0 μg/ml). Sterile distilled water served as a negative control. Substances inducing mutations in the corresponding test strains under activation/non-activation conditions were used

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as positive control. In non-activation variants we used ethidium bromide, 2,7-diamino-4,9-dioxy-5,10-dioxo-4,5,9,10-tetrahydro-4,9-diazo pyrene (DIAM, 10 μg per dish, strain TA 98), and sodium azide (5 μg per dish, strain TA 100).

Tubes with 0.7% selective semi-enriched agar were maintained in a water bath at 100°C until melting and then placed in a thermostatic water bath at 45-46°C. The sample in specified dilutions (0.1 ml) was put to tubes and mixed with 0.1 ml bacterial suspension and 0.5 ml microsomal activation medium (metabolic activation variant). The tubes were removed from water bath during this procedure. The tubes were vigorously agitated and put on the bottom layer of minimal agar in Petri dishes. The dishes were kept at room temperature for 30-40 min and then in a thermostat at 37°C for 48-72 h. Experiments were performed on variants with and without SMA. Each control and treated variant was studied in 3 dishes. The results were considered when the mutagenic effect developed in each variant of the positive control. Statistical treatment included multiple comparison by means of Dunnet test [4].

Preparation-induced chromosomal aberrations (CA) in bone marrow cells were studied on hybrid (CBA× C57Bl/6J)F_1 mice aging 2 months and weighing

TABLE 1. Ability of KPA-Ca to Induce Gene Mutations in Indicator Bacterial Strains (Ames Salmonella/Microsomes Test)

Strain, variant	Preparation	Dose, μg per dish	Number of revertants per dish, X_i	Geometric mean X_i	Statistical value for deviating observations	Ratio for excess
TA 98, no SMA	KPA-Ca	0.0	22; 24; 20	21.94	1.06	1.00
		0.1	11; 12; 18	13.34	1.35	0.61
		1.0	19; 17; 14	16.54	1.18	0.75
		10.0	14; 11; 21	14.79	1.42	0.67
		100.0	20; 21; 23	21.30	1.08	0.97
		1000.0	0; 0; 0	0	—	0
	DIAM	0.0	13; 24	17.61	1.36	1.00
		10.0	99; 79	88.44	1.12	5.02
	Ethidium bromide	0.0	22; 24; 20	21.94	1.06	1.00
		5.0	31; 24	27.28	1.14	1.24
TA 98+SMA	KPA-Ca	0.0	15; 13; 16	14.61	1.12	1.00
		0.1	18; 12; 14	14.46	1.24	0.99
		1.0	12; 9; 12	10.90	1.12	0.75
		10.0	17; 32; 14	19.67	1.63	1.35
		100.0	14; 16; 11	13.51	1.23	0.92
		1000.0	18; 20; 13	16.73	1.29	1.14
	Ethidium bromide	0.0	15; 13; 16	14.61	1.12	1.00
		5.0	478; 524	500.47	1.05	34.12
TA 100,	KPA-Ca	0.0	89; 82; 63	77.18	1.23	1.00
no SMA		0.1	78; 91; 73	80.32	1.13	1.04
		1.0	84; 71; 64	72.54	1.16	0.94
		10.0	80; 61; 96	77.67	1.27	1.01
		100.0	59; 72; 61	63.75	1.13	0.83
		1000.0	0; 0; 0	0	—	0
	Sodium azide	0.0	89; 82; 63	77.18	1.23	1.00
		5.0	1517; 1376	1444.78	1.05	18.72
TA 100+SMA	KPA-Ca	0.0	75; 68; 86	75.98	1.13	1.00
		0.1	78; 84; 82	81.29	1.04	1.07
		1.0	76; 71; 70	72.29	1.05	0.95
		10.0	54; 72; 63	62.57	1.16	0.82
		100.0	73; 64; 52	62.40	1.20	0.82
		1000.0	53; 50; 51	51.32	1.03	0.68

20-22 g. Each control and experimental group consisted of 5 mice. KPA-Ca in a dose of 34 mg/kg was given 1 or 4 times, which corresponded to the daily therapeutic dose. The maximum dose of KPA-Ca (1700 mg/kg) exceeded its therapeutic dose by 50 times. The preparation was *ex tempore* dissolved in distilled water (0.2 ml/20 g). Control animals received an equivalent volume of distilled water. Bone marrow cells were fixed 24 h after treatment.

Colchicine in a dose of 0.01 ml/kg (0.025%) was injected intraperitoneally 2.0-2.5 h before euthanasia to induce metaphase accumulation. The femoral bone marrow was isolated from sacrificed mice and routinely treated for microscopic examination. Preparations were stained with azure by the method of Romanovsky. We examined 100 metaphases in each male from various groups. The study was performed with metaphase plates of regular shape characterized by good spread of chromosomes and having a module number of 40. The results of experimental and control series were compared using χ^2 test.

Embryotoxicity and teratogenicity of the preparation were studied on female (AMCY×Wistar)F_1 rats and male Wistar rats weighing 300-350 and 500-600 g, respectively. The animals fed a standard diet and had free access to water.

To obtain females with known stage of pregnancy, they were housed together with males in the evening. Vaginal smears were examined in the morning. The day when spermatozoa were found in the smear was considered to be the 1st day of pregnancy. The animals were sacrificed on day 20 of pregnancy (immediately before labor). Internal organs were visually examined; viable fetuses, fetal resorption sites, and implantation sites were counted. The placenta and embryos were weighted. The craniocaudal size was measured. The fetus/placenta weight ratio was calculated. The number of corpora lutea was determined. Fetuses were examined by the method of Wilson and Dawson. The observation over some pregnant females (5 specimens of each group) continued until spontaneous labor. We estimated the number of rat pups and studied their postnatal development. The animals were examined 1 month after birth to reveal possible damage to the central nervous system. The behavior of rats was studied in the open field, hole-board test, and grid test. The number of horizontal and vertical ambulations per unit time was calculated on an actinograph.

The results were analyzed by methods by variational statistics (χ^2 test, Fischer—Student's test with Strelkov's modification).

RESULTS

S. typhimurium mutation test is a bacterial test system for studying histidine prototrophy mutations under the influence of chemical compounds and/or their metabolites inducing mutations (base-pair substitution and frame shift). If the test compounds and/or metabolites possess mutagenic activity, they would induce reverse mutations in *S. typhimurium* strains from histidine auxotrophy to prototrophy. The effect of direct mutagens was studied in the non-SMA variant. The mutagenic effect of these compounds is associated with the

TABLE 2. Embryotoxic and Teratogenic Effects of KPA-Ca (*M*±*m*)

Parameter		Control	KPA-Ca, mg/kg	
			40	80
Number of females		16	12	16
Number of corpora lutea	total	192	147	189
	per female	12.0±1.5	12.2±3.8	11.8±2.2
Number of implantation sites	total	169	135	173
	per female	10.6±1.2	11.2±3.1	10.8±1.1
Number of resorption sites	total	23	14	9
	per female	1.43±0.47	1.16±0.2	0.56±0.3
Weight of fetus, mg		3372±230	3425±341	3154±132
Craniocaudal size, mm		35.4±1.4	35.8±1.2	33.6±1.2
Fetus/placenta weight ratio		7.4±2.3	7.5±1.5	6.5±1.1
Preimplantation death, %		11.9	8.20	8.46
Postimplantation death, %		13.6	10.3	5.2
Normal/abnormal	Dawson	73/0	60/0	82/0
	Wilson	73/0	61/0	82/0

TABLE 3. Physiological Characteristics and Behavioral Reactions of Rat Pups from Females Receiving KPA-Ca (*M*±*m*, *n*=25)

Parameter	Control	KPA-Ca, mg/kg	
		40	80
Period of hair formation, days	9.72±1.20	9.76±0.96	9.2±1.1
Opening of eyes, days	16.68±0.32	13.55±0.21	12.90±0.43
Body weight on day 30, g	98.0±5.3	97.5±3.8	91.4±5.9
Number of transitions over 1 min	39.3±7.4	36.2±4.9	37.6±5.3
Number of explored holes (open field)	11.7±4.3	13.4±4.2	19.3±8.1
Number of animals fallen down in the grid test	5	3	5

initial chemical structure. The effects of promutagens related to the formation of mutagenic metabolites were determined by comparing the results of experiments with both variants (with and without the activation mixture). KPA-Ca in doses of 0.1-1000.0 μg per dish exhibited no mutagenic activity in the Ames Salmonella/microsomes test.

Cytogenetic activity of KPA-Ca in mouse bone marrow cells was evaluated by studying the following types of CA: fragments (single and paired) and exchanges. The cell was considered to have multiple CA, when it contained more than 3 CA. Achromatic gaps were assayed individually; they were not included into the total number of CA. Administration of KPA-Ca in specified doses did not increase the count of CA in mouse bone marrow cells under various experimental conditions.

The study of possible embryotoxic effect showed that treated females do not differ from control animals in the total and mean numbers of corpora lutea, implantation sites, and resorption sites. Pregnant rats receiving KPA-Ca and physiological saline did not differ in pre- and postimplantation death, average weight of living fetuses, average craniocaudal size, and average fetus/placenta weight ratio (Table 2). Autopsy showed that fetuses from different groups were viable. These data indicate that KPA-Ca does not modulate pre- and postimplantation stages of embryogenesis.

Viability of fetuses was studied to reveal potential teratogenicity of KPA-Ca. We examined fetuses of female rats receiving KPA-Ca in a dose of 40 (*n*=164) or 80 mg/kg (*n*=121) and control animals (*n*=146). Macroscopic examination by the method of Wilson and Dawson revealed no abnormalities in fetuses of different groups (Table 2).

Some control females and rats receiving KPA-Ca in a dose of 40 or 80 mg/kg were examined after spontaneous labor. These female groups produced 46 (9.2±2.1 per female), 47 (9.4±1.7 per female), and 49 rat pups (9.8±3.2 per female), respectively. Administration of KPA-Ca had no effect on the fertility of females. The development of rat pups did not differ from normal. The weight gain and behavioral reactions in treated rats were similar to those in control animals (Table 3).

Our results show that long-term oral administration of KPA-Ca produced no mutagenic, embryotoxic, and teratogenic effects. The preparation has no embryotoxic and teratogenic properties and, therefore, can be used during pregnancy.

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