GENETICS

Damage to Cell DNA in the Bone Marrow and Testes of Mice with Experimental Trichinosis

Vl. Ya. Bekish and A. D. Durnev*

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Metabolites of *Trichinella spiralis* produced genotoxic and cytotoxic effects on somatic and generative cells of the host organism. They increased the number of single-chain breaks, alkaline-labile sites in nuclear DNA, and count of apoptotic cells in the bone marrow and testes of infected mice. These effects depended on the stage of parasite development in the host organism and became more pronounced with increasing invasion intensity.

Key Words: *Trichinella; alkaline single cell gel electrophoresis; cells of bone marrow and testes; genotoxicity; cytotoxicity*

Trichinella metabolites increase the number of somatic cells with micronuclei and chromosomal aberrations [4] and the count of micronuclear spermatogonia, spermatocytes, and spermatid in the testes of animals and suppress spermatogenesis [2]. Cytogenetic changes in host somatic and generative cells during trichinosis depend on the infective dose of invasive material and correspond to high biological activity of parasites [2,4].

Here we studied the ability of *Trichinella spiralis* metabolites to induce DNA breaks and apoptosis in somatic and generative cells of mice invaded with trichinella depending on the stage of parasite development in the host organism and severity of invasion.

MATERIALS AND METHODS

Experiments were performed on 140 male CBA mice weighing 16-18 g. The animals were divided into 4 groups of 35 mice each. Intact controls (group 1) perorally received 0.2 ml 2% starch gel. Other animals were intragastrically infected with *T. spiralis* larvae in doses of 5, 20, and 40 parasites per 1 g body weight to produce mild, moderate, and severe trichinosis, respectively [1]. The control and infected mice were decapitated on days 3, 7, 14, 21, 28, 60, and 90 after invasion (5 animals in each period). The femur and testes were isolated. The bone marrow was washed with 2 ml RPMI-1640 medium.

The spermatic duct was washed with 5 ml 2.2% sodium citrate using a porcelain mortar. The solution was removed. The spermatic duct was ground with a curved preparation needle in 3 ml freshly prepared sodium citrate and placed in tubes. Tubes were maintained at room temperature for 5 min until precipitation of large fragments. The upper layer was transferred into another tube. The suspension of cells from the bone marrow and testes was washed 3 times with 3 ml RPMI-1640 medium at 1100 rpm and 8°C for 10 min. The pellet was dissolved with RPMI-1640 medium to a final concentration of $1\text{-}5\times10^6$ cells per 1 ml. RPMI-1640 medium (200 μl) was added to the cell suspension from control animals (100 μl). The mixture was incubated with 100 μM H_2O_2 at 37°C for 5 min (positive control). The remaining cells served as the negative control.

The alkaline single cell electrophoretic assay [11] with modifications [6] allows evaluating the number

Department of Medical Biology and General Genetics, Vitebsk State Medical University; *Laboratory for Pharmacology of Mutagenesis, Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow. ***Address for correspondence:*** bekishvl@tut.by. Vl. Ya. Bekish

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of single-chain breaks and alkaline-labile sites in nuclear DNA under the influence of genotoxic factors. The assay was performed in an electrophoresis chamber with a power unit using Sigma reagents. Micropreparations were stained with ethidium bromide and examined under a Mikmed-2 luminescence microscope (LOMO, ×600). Comet images in micropreparations were photographed with a Nikon Coolpix-4500 digital camera. Damage to the DNA molecule was studied in digital images [7]. We examined 100 cells. The length of the comet tail (pixels) and the percentage of tail DNA were estimated (Fig. 1, *a*, 1, 2; *b*, 1, 2). The tail length was multiplied by the tail DNA percentage to calculate the tail moment, which serves as the general international criterion for the genotoxic effect of environmental factors [6,12]. The percentage of apoptotic cells in 100 randomly selected cells was calculated to evaluate the cytotoxic effect of trichinella metabolites on cells of the bone marrow and testes (Fig. 1, *a*, 3; *b*, 3). These cells have the smallest nucleus and "scattered" large tail [5]. The results were analyzed by means of Statistica 6.0 software. The data were expressed as the arithmetic mean and standard deviation ($M \pm SD$).

RESULTS

Trichinella metabolites produced a genotoxic effect on host somatic and generative cells. They *in vivo* increased the number of single-chain breaks and alkaline-labile sites in nuclear DNA of cells in the bone marrow and testes (Table 1). Damage to nuclear DNA in cells of the bone marrow and testes during trichinosis depended on the stage of parasite development. In the intestinal phase of invasion (day 7) trichinella metabolites produced a genotoxic effect on bone marrow cells (moderate trichinosis) or had a combined action on the bone marrow and testes (severe trichinosis). The genotoxic effect of metabolites on host somatic and generative cells was most pronounced at the stage of migration and encapsulation of trichinella larvae (days 14-21 and 28, respectively). Damage to nuclear DNA in cells of the bone marrow and testes depended on the infective dose of invasive material. The severity of damages increased with increasing the dose of parasitic cells. The dose-depended effect was observed in studying changes of the tail moment. Increasing the infective dose from 20 to 40 larvae per 1 g was accompanied by changes in the bone marrow (days 7, 14, 21, and 28) and testes (days 14, 21, and 28). On day 14 of invasion the tail moment in the testes increased by 2.7 and 2.2 times with increasing in the infective dose from 5 to 20 and 40 larvae per 1 g, respectively.

Trichinella metabolites also produced the cytotoxic effect on host somatic and generative cells. It manifested in increased number of apoptotic cells in the bone marrow and testes of infected animals. The effect depended on the stage of trichinella development. During the intestinal phase of invasion (day 7) the count of apoptotic cells in the bone marrow and testes increased only in mice with severe trichinosis. Trichinella metabolites in different infective doses produce a cytotoxic effect during the migration phase of invasion (days 14-21) and encapsulation of parasites (day 28). The increase in the number of apoptotic cells in the bone marrow and testes of mice with trichinosis directly depended on the infective dose of invasive material. The tail moment in the testes (day 14) and bone marrow (days 14 and 21) increased by 1.3-1.9 times with increasing the infective dose from 5 to 20 larvae per 1 g. The dose-dependent cytotoxic

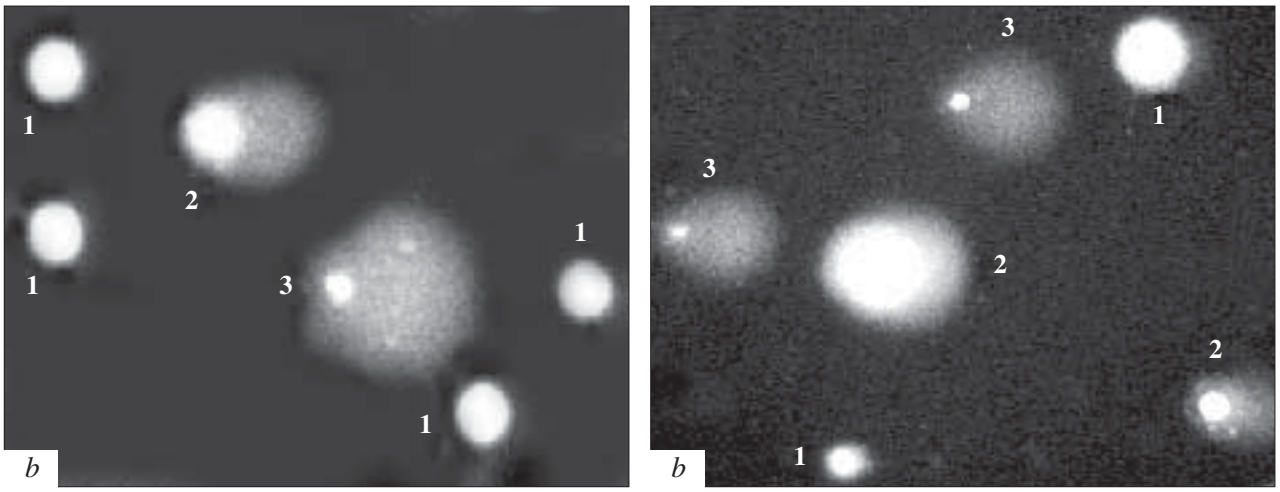

Fig. 1. Comets of cells in the bone marrow (*a*) and testes (*b*) during alkaline single cell electrophoresis. Ethidium bromide staining, ×600. Normal cells (*a*), cells with 8-12% DNA damage in the comet tail (*b*), and apoptotic cells (*c*).

TABLE 1. Alkaline Single Cell Electrophoretic Assay of Cells from the Bone Marrow and Testes of Male CBA Mice with Trichinosis Infected with Different Doses of Invasive Material (*M*±*SD*)

Group		Bone marrow cells		Testicular cells	
		tail moment	percentage of apoptotic cells	tail moment	percentage of apoptotic cells
Day 3	negative control	0.28±0.12	1.60±0.34	0.56±0.18	7.00±1.75
	positive control	3.48±1.18*	5.60±1.90*	7.12±1.86*	10.10±1.52*
	5 larvae/g	0.57±0.18	1.40±0.67	0.56±0.16	6.10±0.74
	20 larvae/g	0.49±0.15	2.30±0.75	0.62±0.13	5.10±1.20
	40 larvae/g	0.59±0.15	1.30±0.48	0.59±0.14	6.40±1.43
Day 7	negative control	0.39±0.14	1.40±0.65	0.43±0.19	6.20±1.55
	positive control	2.35±1.90*	3.60±0.70*	8.68±1.99*	9.10±1.37*
	5 larvae/g	0.72±0.13	1.56±0.48	0.48±0.19	6.22±0.97
	20 larvae/g	1.29±0.70*	2.33±0.51	0.60±0.19	5.67±1.41
	40 larvae/g	2.95±0.81*^{o}	4.56±0.88*	3.62±1.54*	8.00±1.41*
Day 14	negative control	0.35±0.21	1.90±0.74	0.55±0.25	4.80±0.63
	positive control	3.76±1.18*	3.50±0.53*	10.78±2.86*	7.20±1.40*
	5 larvae/g	1.34±0.45*	1.90±0.88	0.80±0.12*	7.90±0.99*
	20 larvae/g	1.47±0.55*	3.10±0.74*	2.19±0.47*$^{+}$	10.30±1.49*$^{+}$
	40 larvae/g	4.50±1.30*^{o}	6.50±0.85*^{o}	4.85±0.97*^{o}	18.10±2.02*^{o}
Day 21	negative control	0.45±0.23	1.20±0.53	0.59±0.15	4.50±1.27
	positive control	4.17±1.56*	4.40±0.84*	10.06±2.99*	8.20±1.69*
	5 larvae/g	0.22±0.31	2.80±0.92*	0.64±0.16	7.20±1.23*
	20 larvae/g	1.32±0.35*	5.30±0.95*$^{+}$	1.83±0.32*	12.00±1.41*$^{+}$
	40 larvae/g	4.96±1.99*^{o}	5.20±0.92*	4.67±1.02*^{o}	17.60±2.41*^{o}
Day 28	negative control	0.13±0.13	2.00±0.67	0.43±0.11	4.90±1.20
	positive control	5.26±1.21*	4.70±0.67*	10.54±4.62*	8.80±1.48*
	5 larvae/g	0.32±0.15	2.50±0.45	0.55±0.13	5.40±1.51
	20 larvae/g	0.65±0.12*	4.50±1.58*	1.44±0.28*	7.40±0.84*
	40 larvae/g	3.02±0.44*^{o}	7.00±0.82*^{o}	4.09±0.57*^{o}	12.60±1.17*^{o}
Day 60	negative control	0.35±0.12	2.20±1.03	0.70±0.26	5.70±0.95
	positive control	4.49±2.01*	4.50±1.27*	10.82±4.59*	8.10±1.60*
	5 larvae/g	0.20±0.21	2.40±1.35	0.45±0.12	6.10±1.20
	20 larvae/g	0.42±0.13	1.50±0.53	0.77±0.15	5.10±0.74
	40 larvae/g	0.46±0.19	1.80±1.32	0.72±0.17	6.10±1.20
Day 90	negative control	0.21±0.17	2.30±0.82	0.54±0.14	5.50±1.08
	positive control	6.12±2.36*	4.30±1.16*	6.76±1.78*	7.90±2.60*
	5 larvae/g	0.44±0.24	1.50±0.45	0.45±0.14	5.50±0.85
	20 larvae/g	0.45±0.19	1.80±0.19	0.38±0.19	4.60±1.17
	40 larvae/g	0.31±0.12	2.00±0.67	0.50±0.12	4.90±0.74

Note. p<0.01-0.05: *compared to the control; $^{+}$compared to 5 larvae/g; ^{o}compared to 20 larvae/g.

effect of trichinella metabolites was observed in the bone marrow (days 14 and 28) and testes (days 14, 21, and 28) with increasing the infective dose from 20 to 40 larvae per 1 g.

The genotoxic and cytotoxic injury in the bone marrow and testes of infected animals was probably due to the development of oxidative stress in the host organism and ability of metabolites to damage the nuclear apparatus in host cells. Our previous studies showed that invasion with trichinella is accompanied by activation of free radical processes in the muscles and testes of mice. We observed an increase in the content

of lipid peroxidation products (malonic dialdehyde and conjugated dienes) and decrease in activity of antioxidant enzymes (superoxide dismutase and catalase) [3]. Moreover, secretory and excretory products of trichinella larvae include single-chain and two-chain endonucleases [8,9] and DNA-twisting proteins [10].

Our results suggest that bioactive trichinella metabolites produce genotoxic and cytotoxic effects on host somatic and generative cells. They increased the number of single-chain breaks, alkaline-labile sites in nuclear DNA and count of apoptotic cells in the bone marrow and testes. This effect was maximum in the intestinal (severe trichinosis) and migration phase of disease (mild, moderate, and severe trichinosis). The genotoxic and cytotoxic effect of trichinella metabolites on host somatic and generative cells depended on the severity of invasion and increased with increasing the infective dose of the invasive material.

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