Chromosomal Aberrations and Spermhead Abnormalities Induced by Abamectin (Avermectin B₁) and its Degradates in Male Swiss Albino Mice

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Accepted October 30, 1995

Avermectins are a complex of chemically related naturally occurring macrocyclic lactones which first isolated as a natural fermentation product of a soil actinomycete, *Streptomyces avermitilis*, in 1976 (Burg *et al.* 1979). The naturally occurring avermectins can be separated into four homologous pairs, of which one, abamectin (avermectin B₁), which consists of 80% of avermectin B_{1a} and 20% of avermectin B_{1b} has been developed for use against arthropod pests of horticulture and agronomic crops (Dybas and Green 1984). Currently, it is rapidly worldwide growing in usage for many agronomic crops. They are potent chemotherapeutic agents against animal parasitic nematodes and many veterinary and agricultural arthropod pests (Putter *et al.* 1981, Campbell *et al.* 1983, Lasota and Dybas 1991). Abamectin increases calcium permeability to chloride ions, which is independent of the neurotransmitter, gamma-aminobutyric acid (GABA)-mediated chloride ion channels (Calcott and Fatig 1984). The compounds' mechanism of toxicity in mammals is unknown but GABA is a mammalian central nervous system neurotransmitter and effects on GABA may be relevant to their safety in mammals (Lankas and Gordon 1989). Carcinogenicity studies with abamectin at maximum tolerated dosage levels for 22 and 24 months duration in mice and rats, respectively, have demonstrated the lack of carcinogenicity of this compound (Lankas and Gordon 1989). Also they showed the lack of genotoxic activity of abamectin in a variety of assays. Abamectin degradates were tested and found to have no adverse maternal and developmental effects in CF 1 mouse, the laboratory species/strain most sensitive to abamectin toxicity. In addition, the degradates from photolysis of thin films were negative in the microbial mutagenesis assay (Crouch *et al.* 1992).

The specific objectives of the present study were; (1) to estimate the percentage of mitotic abnormalities in male mice bone marrow cells induced by abamectin and its degradates, (2) to measure the amount of spermhead abnormalities in male mice caused by abamectin.

Materials and methods

Chemical

Commercially available sample of abamectin, as 'Avermectin B₁', 1.8% wt/vol emulsifiable concentrate, produced by Merck, Sharp & Dohme, Three Bridge, N.J., USA., was used in the bioassays. The chemical structure is a mixture of homologous products with a minimum of 80% B_{1a} and a maximum of 20% B_{1b} components. The B_{1b} component differs chemically from the B_{1a} component by only 1 methylene (CH₂) unit at the 26-carbon position; the ethyl group (C₂H₅) is a methyl group in the B_{1b} form. The compound's dilutions were made in distilled water.

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Animals and Experimental Procedure

Animal: Male swiss albino mice, *Mus musculus*, were obtained from a laboratory colony and kept until they reached an age of 8–10-weeks, weighing 22–25 g each, before being used.

Bone Marrow Cell Suspension Preparation: Male mice were chosen randomly from the colony and divided into three groups. Mice were injected with 0.5 ml diluted abamectin solutions using acute intraperitoneal (i.p.) administration procedure. Where animals in the first and second groups were injected with the doses and 1 of 3 $\mu\text{g/g}$ body weight (b.w.) that selected on the basis of 1/30 and 1/10 LD_{50} value, respectively (Lankas and Gordon 1989). Third group served as control, at which mice were injected i.p. with 0.5 ml of distilled water. The mice in each group, were injected with colchicine (0.6 mg/kg) at one, two, five and eight days after treatment and kept for 2 hr, then the animals were sacrificed by cervical dislocation. Both femurs were immediately removed and femoral contents were flushed with phosphate saline (136.9 mM NaCl, 2.68 mM KCl, 8.09 mM NaHPO_4 and 1.47 mM H_2PO_4) into a small tube by using small syringe needle (26 gauge). The cell suspension was centrifuged at 1000 r.p.m. for 5 min. A hypotonic solution (0.075 M KCl) was added to cell pellet and let stand for 12 min at 37°C, then centrifuged. For final fixation, about 1–2 ml fresh fixative (methanol:acetic acid, 3:1 v/v) was added to cell pellet for 10 min, and slides were prepared according to Scribner *et al.* (1983) and stained with 4% Giemsa before examination by light microscope. The chromosomal aberrations induced by selected doses of abamectin were recorded and photomicrographed at 1000-fold magnification. Three animals were used for each treatment, and about 70 metaphase plates per animal were examined.

Sperm Suspension Preparation: Male mice were injected intraperitoneally with the same doses used previously in bone marrow assays. Male mice were sacrificed at 1, 3, 5, and 8 weeks after treatment and their caudal epididymides were removed. Three sperm suspensions were prepared from the two cauda of each mice as described by Wyrobek and Bruce (1975). A fraction of each suspension was then mixed (10:1) with 1% Eosin Y (H_2O) and 30 min later air dried smears of sperm suspension were made. Sperms were examined at 400-fold magnifications with blue-green filters. Three animals were examined for each assay.

Preparation of abamectin degradates

0.15 mg of the commercial abamectin was applied to a glass beaker to give a thin film of approximately $9.6 \mu\text{g}/\text{mm}^2$ after drying; this amount is equivalent to 1/4 LD_{50} value (Lankas and Gordon 1989) for a mice weighing 20 g. Three beakers were used for each treatment. Such beakers were left outdoor to be exposed to sunlight during the day and to the natural environment at night. The temperature was 35–37°C during the day and it was 25–27°C, at night. Thin film residues were recovered by rinsing in a 0.5 ml of distilled water, after 1, 2, 3, 4, 5 and 6 days of exposing. Male mice were injected i.p. with the six collected residues of abamectin. Another group of animals was served as control at which mice were injected i.p. with 0.5 ml of distilled water. Mice in each group were injected with colchicine (0.6 mg/kg) at 24 hr posttreatment. Finally, they were sacrificed and their femurs were removed for bone marrow cell suspension preparations as described previously.

Results and discussion

Mitotic Abnormalities

Untreated bone marrow cells of male Swiss albino mice recorded in metaphase showed $2n=40$ (Fig. 1). Injection of male mice with abamectin or its degradates induced mitotic aberrations which were categorized into two groups, physiological and clastogenic aberrations. Aberrations which may be explained by chromosomal/chromatid breaks or their consequences

Figs. 1-9. Bone marrow cells (BMC) from male Swiss albino mice. 1, a BMC showing a normal complement of 40 chromosomes from untreated mice. 2, a BMC showing a ring chromosome. 3, a BMC showing an acentric fragment. 4-a, a BMC showing a chromatid gap. 4-b, a BMC showing a chromosomal gap. 5, a BMC showing a terminal deletion. 6, a BMC showing centric association. 7, a BMC showing polycentric association. 8, a BMC showing chromosomal stickiness. 9, a BMC showing polyploidy (4n).

were categorized as clastogenic abnormal. On the other hand, abnormalities which may be due to effects of other than chromosome/chromatid breaks or their consequences were categorized as physiological abnormalities (Grover and Malhi 1988).

(a) *Clastogenic Abnormalities*: No clastogenic metaphases was observed in the control (untreated) mice. Injection of male mice with either low or high dose of abamectin resulted in significant increase in the number and percentage of clastogenic metaphases than that of control which included; ring chromosomes (Fig. 2), fragmentation (Fig. 3), chromatid gaps (Fig. 4-a), chromosome gaps (Fig. 4-b), and terminal deletion (Fig. 5). The percentage of aberrant metaphases ranged from 6.5 to 18%, at low dose, and from 8.5 to 16.5%, at the high dose (Table 1). Such type of clastogenic aberration induced in bone marrow cells was reported by other researchers (Georgion 1975, Sharma and Guatma 1991, Manna and Mohanty 1993), which may be possibly produced from direct interaction between abamectin and chromosomal DNA. The observed ring chromosomes could be resulted from rejoining of two broken ends of the same chromosome to form a ring, consequently; the frequency of fragments was high as

Table 1. Bone marrow clastogenic abnormalities at four intervals in male Swiss albino mice injected intraperitoneally (i.p.) with abamectin (Avermectin B₁)

Dose ($\mu\text{g/g}$ b.wt. i.p.)	Post- treatment interval (in days)	Total number of observed metaphases	Number of abnormal clastogenic metaphases	% Abnormal ^a clastogenic metaphases	Number of clastogenic abnormalities ^b				
					Rc	ChFr	ChGa	ChtdGa	TchDe
1.0	1	200	36	18.0 a	10	21	1	3	1
	2	200	26	13.0 bc	9	6	1	6	4
	5	200	21	10.5 c	5	11	0	3	2
	8	200	13	6.5 d	2	9	0	1	1
3.0	1	200	33	16.5 ab	6	19	2	3	3
	2	200	34	17.0 ab	13	8	1	6	6
	5	200	23	11.5 c	6	10	0	4	3
	8	200	17	8.5 cd	6	9	0	2	0
Control	1	200	0	0 e					
	2	200	0	0 e					
	5	200	0	0 e					
	8	200	0	0 e					

^aValues within a column followed by the same letter(s) are not significantly different at the $P=0.05$ level according to the Least Significant Difference Test (Snedecor and Cochran 1967). ^bRC, ring chromosomes; ChFr, chromosomal fragments; ChGa, chromosomal gaps; Chtd Ga, chromatid gaps; TchDe, terminal chromatid deletion.

compared to ring chromosomes (Table 1). Formation of chromosome and chromatid gaps suggests that abamectin interacts DNA independently of the cell cycle stage. At the low dose of abamectin-treatment, the highest percentage of clastogenic metaphases (18%) was shown after 24 hr, whereas, at the high dose, the highest percentages (16.5 and 17%) were shown after 24 and 48 hr of treatment, respectively. It seems that, the highest percentage of clastogenic metaphases appeared to decrease significantly, at 1 and 2 days, reaching 6.5% and 8.5% after 8 days of treatment, for the low and high dose used, respectively. This significant decrease in the chemical effect may be due to the accumulation effects of the amount of lesions repaired by the mice's enzyme system.

(b) *Physiological Abnormalities*: The physiological effects observed after treatment with abamectin were; centric association (Fig. 6), polycentric association (Fig. 7), stickiness (Fig. 8) and polyploid (Fig. 9). The percentages of abnormal metaphases ranged from 9 to 13.5%, at the lower dose of $1.0 \mu\text{g/g}$ b.w., and from 7.5 to 15.5%, at the higher dose of $3.0 \mu\text{g/g}$ b.w. (Table 2). The percentages of aberration metaphases were found to be high and different significantly than that of the control, at both doses used, at 1, 2, 5, and 8 days after treatment. These abnormalities may be ascribed to cellular disturbances in the spindle organizations (Grover and Malhi 1988).

Spermhead Abnormalities

The eosin stained smear of epididymes sperm of male mice showed various types of spermhead abnormalities after one week of treatment only (Table 3). The observed abnormalities in the spermhead were happened during spermatogenesis because once the spermhead develops its shape, it is extremely stable (Brusick 1980). Since the animals were killed at 1, 3, 5, and 8 weeks after injection with abamectin, the sperms derived from cells, that exposed during spermatogenesis; thus the sperm samples were as epididymal sperm, spermatides, spermatocytes, and early stages of meiosis (Bateman and Epstein 1971). Fig. 10 illustrates normal and abnormal spermheads which were obtained after one week of treatment. The high percentage of abnormal spermheads observed after one week of treatment was significantly

Table 2. Frequencies of physiological abnormal metaphases at four intervals in male Swiss albino mice injected intraperitoneally (i.p.) with abamectin (Avermectin B₁)

Dose (μ g/g b.wt. i.p.)	Post-treatment interval (in days)	Total number of observed metaphases	Number of abnormal physiological metaphases	% Abnormal ^a physiological metaphases	Number of physiological abnormalities ^b				
					St	Ca	Pca	Polyploidy	
								4n	8n
1.0	1	200	18	9.0 cd	13	2	2	0	1
	2	200	24	12.0 bc	9	3	2	1	9
	5	200	27	13.5 ab	14	9	0	0	3
	8	200	17	8.5 cd	8	7	0	0	2
3.0	1	200	15	7.5 d	12	1	2	0	0
	2	200	31	15.5 a	10	5	3	1	12
	5	200	19	9.5 cd	10	5	1	0	3
	8	200	23	11.5 bcd	10	8	1	0	4
Control	1	200	3	1.5 e	3	0	0	0	0
	2	200	0	0.0 e	0	0	0	0	0
	5	200	2	1.0 e	2	0	0	0	0
	8	200	3	1.5 e	3	0	0	0	0

^a Values within a column followed by the same letter(s) are not significantly different at the $P=0.05$ level according to the Least Significant Difference Test (Snedecor and Cochran 1967). ^b St, stickiness; Ca, centric association; Pca, polycentric association.

Table 3. Frequencies of abnormal spermhead at five intervals in male Swiss albino mice injected intraperitoneally with abamectin (Avermectin B₁)

Dose (μ g/g b.w. i.p.)	Total number of sperms scored	Number of different abnormal spermhead types ^{a,b}							
		LkH	BaL	Fl	LnH	Am	Others	Total	%
1-week interval									
1.0	616	81	20	158	27	95	22	403	65.4 a
3.0	878	37	33	47	9	44	20	190	21.6 b
Control	1021	8	4	13	6	11	1	43	4.2 c
3-week interval									
1.0	2000	44	5	15	11	21	12	108	5.4 c
3.0	2000	24	3	26	11	28	11	103	5.2 c
Control	1000	10	2	6	1	10	4	33	3.3 c
5-week interval									
1.0	2000	14	2	11	6	9	4	46	2.3 c
3.0	2000	18	4	21	4	14	4	65	3.3 c
Control	1000	7	1	6	2	5	1	22	2.2 c
8-week interval									
1.0	2000	20	4	17	8	2	15	66	3.3 c
3.0	2000	25	15	14	10	7	20	91	4.6 c
Control	1000	7	2	7	3	1	2	22	2.2 c

^a Values within a column followed by the same letter(s) are not significantly different at the $P=0.05$ level according to the Least Significant Difference Test (Snedecor and Cochran 1967). ^b LkH, lack nook; BaL, banana-like head; Fl, flat; LnH, long hook; Am, amorphous.

different than that of the control. Such high percentage observed, after one week of injection, indicated that the epididymal sperm is the only state affected by abamectin-treatment. This specificity of abamectin may be due to the differences in the capabilities of repair enzyme systems presented in different stages of mice spermatogenesis.

Cytotoxicity of abamectin degrades

Injection of male mice with abamectin degraded for 1, 2 and 3 days induced significant

Fig. 10. Sperms of male Swiss albino mice. a, a sperm with normal head morphology. b, a sperm lack the usual hook. c, a sperm with a banana-like head. d, a sperm with a flat head. e, sperm with a long hook head. f, a sperm with amorphous head. g, other types of abnormal spermhead morphology.

Table 4. Bone marrow clastogenic abnormalities in male Swiss albino injected intraperitoneally (i.p.) with residues obtained from degradation of abamectin thin films for six intervals.

Degradation interval (in days)	Total number of observed metaphases	Number of abnormal clastogenic metaphases	% Abamectin clastogenic metaphases ^a	Number of clastogenic abnormalities ^b				
				RC	ChFr	ChGa	ChtdGa	TchDe
1	200	29	14.5 a	5	22	2	0	0
2	200	13	6.5 b	4	9	0	0	0
3	200	13	4.5 b	2	7	0	0	6
4	200	15	1.5 c	1	1	0	1	0
5	200	3	1.0 c	1	0	1	0	0
6	200	2	0 c	0	0	0	0	0
Control	200	0	0 c	0	0	0	0	0

^a Values within a column followed by the same letter(s) are not significantly different at the $P=0.05$ level according to the Least Significant Difference Test (Snedecor and Cochran 1967). ^b RC, ring chromosomes; ChFr, chromosomal fragments; ChGa, chromosomal gaps; Chtd Ga, chromatid gaps; TchDe, terminal chromatid deletion.

increase in the percentage of clastogenic metaphases than that of the control (Table 4). However, abamectin degraded for 4, 5 and 6 days did not cause any significant increase in the clastogenic metaphases. Such effect obtained under our experimental conditions can be explained that the amount of abamectin applied to each beaker had been degraded into other compounds which did not cause any significant clastogenic metaphases. Our results, are in accord to other results obtained by Crouch *et al.* (1992).

Summary

In this study, several fundamental questions concerning the ability of abamectin (Avermectin B₁) and its degradates to induce chromosomal aberrations in bone marrow cells and spermhead abnormalities in male Swiss albino mice were addressed. Male mice were injected intraperitoneally with two doses of abamectin (i.e. 1/10 and 1/30 LD₅₀, 1.0 and 3.0 $\mu\text{g/g}$ body weight, respectively). One, two, five, and eight days after treatment, males were sacrificed and bone marrow cells slides were prepared for chromosomal studies. Also, after one, three, five, and eight weeks of treatment; other males were sacrificed and sperm suspension slides were prepared for spermhead abnormalities' counting. Abamectin induced clastogenic type of

chromosomal aberrations which significantly decreased by the days following treatment until reached the least after 8 days of treatment. Also, abamectin was found to induce physiological type of aberrations. At the same time, abamectin treatment resulted in high frequency of spermhead abnormalities after one week of injection, which represents the epididymal sperm stage of the animal spermatogenesis. It was found that abamectin which had been degraded for 1–3 days induced significant increase in the percentage of clastogenic metaphases; whereas insignificant effect was observed after 4–6 days.

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