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Carbendazim generates symplasts in rat spermatogenic clones

M A Akbarsha, B Kadalmani*, R Girija & A Faridha

Department of Animal Science, School of Life Sciences, Bharathidasan University, Tiruchirappalli 620 024, India.

*Department of Zoology, Jamal Mohamed College, Tiruchirappalli 620 020, India.

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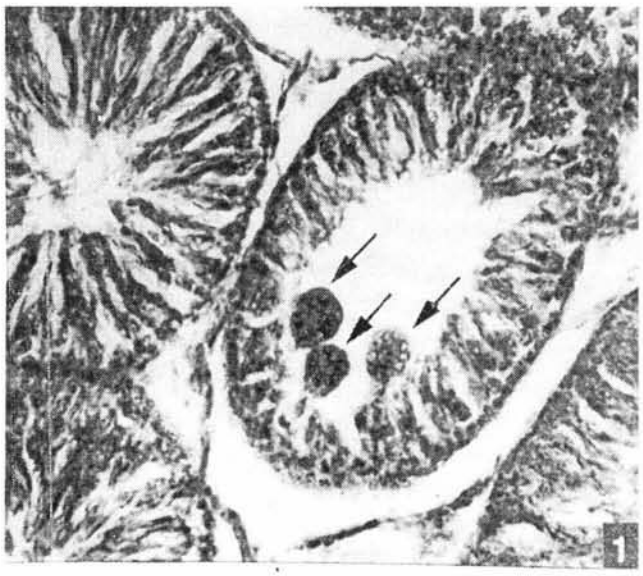
In order to find non-microtubular targets in the seminiferous epithelium for the fungicide and reproductive toxicant carbendazim, it was administered to 90 days old male Wistar rat in a single bolus dose of 400 mg/kg body weight through an oral intubation. A parallel control group was maintained. Rats were sacrificed 48 days after the treatment and the testes were analysed for histopathological changes adopting routine histological methods,when symplasts were localised. The maximum diameter of five largest symplasts was measured, and the number of nuclei in these symplasts was also determined . As it is known that symplasts of spermatogenic cells are produced due to opening up of the intercellular bridges between cells in a clone consequent upon disruption of actin microfilaments, the present study shows that actin microfilaments would also be targets in the seminiferous epithelium for carbendazim toxicity.

Carbendazim (methyl-2-benzimidazole carbamate, MBC) is a fungicide widely used in the agricultural and warehousing practices. Its fungicidal property resides in its ability to disrupt the microtubules[1,2]. Through environmental contamination as well as occupational exposures, man could be exposed to MBC and, thereby, suffer through microtubule disruption. Studies in the laboratory animals have revealed that on exposure to MBC spermatogenesis is impaired through disruption of the microtubules of the spermatogenic cells in division and the Sertoli cell[3-10], causing sloughing of the apical parts of the Sertoli cell along with the associated spermatids[7].In the present study yet another mechanism of action of MBC in causing impairment of spermatogenesis, namely generation of symplasts in the spermatogenic clones,is reported. Symplasts are formed due to disruption of actin microfilaments of intercellular bridges between the cells in a germ cell clone[11].

MBC (*Bavistin*, BASF India Ltd., Bombay) was purchased from a local agrochemical supplier.Three months old Wistar strain male albino rats (20) used in the experiment were fed on standard pellet feed and water *ad libitum*. The experimental group consisted of 10 rats and each rat received a single bolus dose of 400 mg MBC/kg body weight through an oral intubation, according to Nakai *et al.*[12] who have standardized this as the optimal dose for bringing about testicular effects. The control rats of equal number received the oil. The rats were sacrificed 48 days after the treatment and slices of testes were fixed in Bouin's fluid, embedded in paraffin and serial sections at 3 μm thickness were obtained for staining in Harris haematoxylin and eosin. The seminiferous tubules were observed under a Leitz diaplan microscope (Germany) for deformities, and wherever symplasts were found the sections were analyzed critically. The maximum number of symplasts in a section of the seminiferous tubule were counted and the maximum diameter of each of the symplasts was measured using a calibrated ocular micrometer at ×450 magnification. The number of nuclei in the symplast was determined according to Ren and Russell[11]. The total number of nuclei in each of the five largest symplasts was recorded. The data were used to calculate the mean and the standard deviation.

The seminiferous tubules of the control rats had no deformities and did not contain any symplast. On the other hand seminiferous tubules of the MBC-treated rats reflected several deformities. The observation relevant to the present study was occurrence of symplasts in several seminiferous tubules of four of the treated rats (Fig.1). The maximum number of symplasts in section of a tubule was seven. The symplasts were either lying in the lumen or partially embedded in the epithelium, in either case held in position by processes of the Sertoli cell (Fig. 1). The diameter of the largest symplast was 43 μm. The maximum number of nuclei in a symplast was 82 (Table 1). The nuclei, though generally appeared distributed throughout the symplast without any

specific pattern of spatial distribution, in some of the symplasts they were oriented around the periphery of the symplastic mass (Fig. 2). The morphology of the nuclei was typically spermatidic. Sometimes large areas of cytoplasmic continuity were observed between adjacent symplasts (Figs 1 and 2)



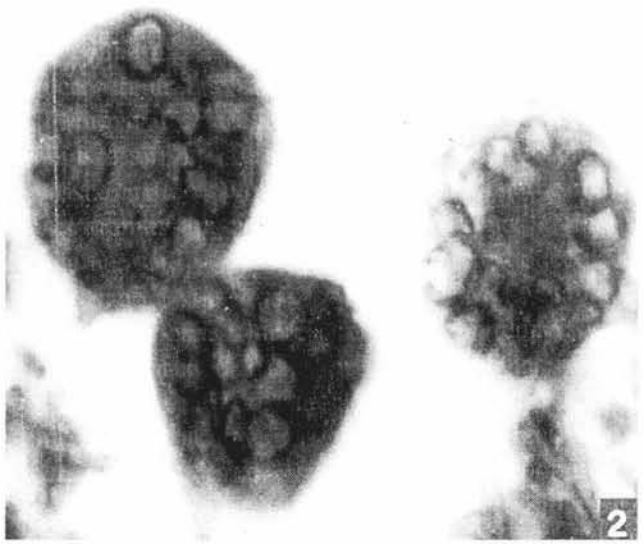


Fig. 1—Section of seminiferous tubules of a carbendazim-treated rat showing symplasts (arrows) (×200).
Fig. 2—Section of three large symplasts in a seminiferous tubule of a carbendazim-treated rat (×1000).

MBC is known to inhibit mitosis in fungi and a variety of eukaryotic organisms through its ability to bind β-tubulin and disrupt the normal formation of microtubule[1,2]. The latter property of MBC is considered responsible for various testicular defects observed in laboratory studies[7].

Testicular damage caused by MBC includes inhibition of testicular microtubule assembly[8,13], failure of poleward movement of chromosomes during spermatocytic division resulting in necrosis of meiotic spermatocytes in Stage XIV tubules, sloughing of immature spermatids[7,8,14] and seminiferous tubular atrophy[6,12,14,16]. MBC induces various morphological abnormalities in round and elongating spermatids, including abnormally formed acrosome[4,9,17] .Origin of large round spermatids (megaspermatids and binucleate spermatids) on MBC treatment has also been reported[4]. Megaspermatids are spermatids with aneuploidy caused due to failure of the microtubule of the spindle[4,18].

Sertoli cell, with its abundant microtubules, also is a target for MBC action, and due to microtubule disruption in the body region the apical portions of the Sertoli cell slough off and such sloughed off Sertoli cell fragments, along with the associated elongating spermatids, arrive at the ductuli efferentes and ductus epididymidis and occlude the lumen of these ducts[7]. Such an occlusion brings about secondary manifestations in the seminiferous tubules resulting in long-term effects, like germinal epithelial cell necrosis and severe inflammatory responses leading to fibrosis of testis[19], to be followed by permanent atrophy of seminiferous tubules and male infertility[12,14,16,19]. In trying to find an interpretation for increased testis weight and accumulation of fluid in seminiferous tubules leading to swelling of the testis after MBC-induced efferent duct occlusion, Nakai *et al.*[12] suggested that non-microtubule targets may also be involved in the MBC mechanism.It was

Table 1—Total number of nuclei and the maximum diameter of five largest symplasts generated in the seminiferous epithelium on treatment with carbendazim

Parameter	Largest symplast					Mean ± SD
	1	2	3	4	5	
Total number of nuclei	82	80	79	76	72	77.8 ± 7.6
Maximum diameter (μm)	42.98	36.84	33.77	33.77	30.70	35.61 ± 8.59

also pointed out that MBC-based disruption of cytoskeletal elements other than microtubules may be important for understanding the effects of MBC[7,10]. Nakai and Hess[7] observed bi- and multinucleate spermatids in MBC-treated rats, and assumed that they are formed by failure of cytokinesis of secondary spermatocytes following nuclear division.

The present study provides evidence for disruption of cytoskeletal elements other than microtubules in the seminiferous epithelium by MBC, as envisaged by Nakai and Hess[7]. The symplasts produced in the seminiferous tubules of MBC- treated rats compare with those produced on treatment with cytochalasin-D[11] and ursolic acid[20]. Spermatidic symplasts are multinucleate cells and formed due to nuclei of all spermatids of a clone joining together as a result of opening of the intercellular bridges[21,22].The intercellular bridges are large areas of cytoplasmic continuity, 1 to 3 μm in diameter[23]. The cells thus connected are considered to be a clone. Presence of intercellular bridges is meant to allow distribution of gene products to all cells of a clone[24]. The intercellular bridges are maintained due to actin microfilaments[12]. Symplasts are formed due to rapid opening up of the intercellular bridges in the germ cell clones in view of actin microfilament disruption[11,22,24]. As individual bridges open the nuclei of all spermatids, at certain Stages of the spermatogenic cycle, go together to form a large multinucleate mass called symplast[21,22].

Therefore, the present study indicates that cytoskeletal elements other than microtubules may also be targets in the spermatogenic compartment of the testis for MBC toxicity,and as seen in the generation of spermatidic symplasts actin microfilaments are one such target.

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References

1 Davidse L C & Flach W, Differential binding of methyl benzimidazol 2-yl carbamate to fungal tubulin as a mechanism of resistance to this antimitotic agent in mutant strains of *Aspergillus nidulans*, *J Cell Biol*, 72 (1977) 174.

2 Burland T G & Gull K, Molecular and cellular aspects of the interaction of benzimidazole fungicides with tubulin and microtubules: In *Mode of Action of Antifungal Agents*, edited by A P J Trinsey and J F Ryley (Cambridge Univ Press, New York), 1984, 300.

3 Tyrkiel E, Investigation of carbendazim(MBC) effect on the spermatogonia of male rats, *Rocz Panstw Zakl Hib*, 35(1984) 161.

4 Nakai M & Hess R A, Effects of carbendazim(methyl 2-benzimidazole carbamate; MBC) on meiotic spermatocytes and subsequent spermiogenesis in the rat testis, *.Anat Rec*, 247 (1997) 379.

5 Parvinen M & Kormano M, Early effects of antispermatogenic benzimidazole derivatives U. 32. 422 E and U.32.104 on the seminiferous epithelium of the rat, *Andrologia*, 6 (1974) 245.

6 Gray L E, Ostby J, Linder R, Goldman J, Rehnberg G & Cooper R,Carbendazim-induced alterations of reproductive development and function in the rat and hamster, *Fundam Appl Toxicol*, 15 (1990) 281.

7 Nakai M & Hess R A, Morphological changes in the rat Sertoli cell induced by the microtubule poison carbendazim, *Tissue Cell*, 26 (1994) 917.

8 Lim J & Miller M G, The role of the benomyl metabolite carbendazim in benomyl-induced testicular toxicity, *Toxicol Appl Pharmacol*, 142 (1997) 401.

9 Nakai M, Hess R A, Natsuo F, Gotoh Y & Nasu T, Further observation on carbendazim - induced abnormalities of spermatid morphology, *Tissue Cell*, 29 (1997) 477.

10 Nakai M, Hess R A, Netsu J & Nasu T, Deformation of the rat Sertoli cell by oral administration of carbendazim (methyl 2-benzimidazole carbamate), *J Androl*, 16 (1995) 410.

11 Ren H P & Russell L D, Clonal development of interconnected germ cells in the rat and its relationship to the segmental and subsegmental organization of spermatogenesis, *Am J Anat*, 192 (1991) 121.

12 Nakai M, Hess R A, Moore B J, Guttroff R F, Strader L F and Linder R E, Acute and long term effects of a single dose of the fungicide carbendazim (methly 2-benzimadole carbamate) on the male reproductive system in the rat, *J Androl*, 13 (1992) 507.

13 Klinefelter G R & Hess R A ,Testis xenobiotic mtabolizing ezymes: In *Reproductive and Developmental Toxicology*, edited by K S Korach (Marcel Dekker Inc., New York) 1998, 553.

14 Hess R A, Moore B J, Forrer J, Linder R E & Abuel-Atta A A, The fungicide benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate) causes testicular dysfunction by inducing the sloughing of germ cells and occlusion of efferent ductules, *Fundam Appl Toxicol*, 17 (1991) 733.

15 Carter S D & Laskey J W, Effect of benomyl on reproduction in the male rat, *Toxicol Lett*, 1(1982) 87.

16 Carter S D, Hess R A & Laskey J W, The fungicide methyl 2-benzimidazole carbamate causes infertility in male Sprague-Dawley rats, *Biol Reprod*, 37(1987) 709.

17 Nakai M, Toshimori K, Yoshinaga K, Nasu T & Hess R A, Spermatids of prepubertal male rats are susceptible to carbendazim during early spermiogenesis, *Arch Histol Cytol*, 61(1998) 433.

18 Evenson D P, Janca F C & Jost L K, Effects of the fungicide methyl-benzimidazol-2-yl carbamate(MBC) on mouse germ cells as determined by flow cytometry, *J Toxicol Environ Health*, 20 (1987) 387.

19 Nakai M, Moore B J & Hess R A, Epithelial reorganization and irregular growth following carbendazim - induced injury of the efferent ductules of the rat testis, *Anat Rec*, 235 (1993) 51.

20 Akbarsha M A , Palanisamy M, Murugaian P & Lakshmi Latha P N, Ursolic acid generates symplasts in rat spermatogenic clones, *Phytother Res*, 12 (1998) 32.

21 Weber J E, Russell L D & Vogl A W, Preliminary studies on cytochalasin D (CD)-related actin filament disruption in rat seminiferous tubules, *J Cell Biol*,101 (1985) 368.

22 Russell L D, Vogl AW & Weber J E, Actin localization in male germ cell intercellular bridges in the rat and ground squirrel and disruption of bridges by cytochalasin D, *Am J Anat*, 180 (1987) 25.

23 Weber J E & Russell L D, A study of intercellular bridges during spermatogenesis in the rat, *Am J Anat*, 180 (1987) 1.

24 Braun R E, Behringer R R, Peschon J J, Brinster R L & Palmiter R D, Genetically haploid spermatids are phenotypically diploid, *Nature*, 337(1989) 373.