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Effects of abamectin exposure on male fertility in rats: Potential role of oxidative stress-mediated poly(ADP-ribose) polymerase (PARP) activation

Ciler Celik-Ozenci [a,*], Arda Tasatargil [b], Merih Tekcan [a], Leyla Sati [a], Ece Gungor [a], Mehmet Isbir [b], Ramazan Demir [a]

[a] Department of Histology and Embryology, Akdeniz University, School of Medicine, Antalya 07070, Turkey
[b] Department of Pharmacology, Akdeniz University, School of Medicine, Antalya 07070, Turkey

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ABSTRACT

Despite the known adverse effects of abamectin pesticide, little is known about its action on male fertility. To explore the effects of exposure to abamectin on male fertility and its mechanism, low (1 mg/kg/day) and high dose (4 mg/kg/day) abamectin were applied to male rats by oral gavage for 1 week and for 6 weeks. Weight of testes, serum reproductive hormone levels, sperm dynamics and histopathology of testes were used to evaluate the reproductive efficiency of abamectin-exposed rats. Abamectin level was determined at high concentrations in plasma and testicular tissues of male rats exposed to this pesticide. The testes weights of animals and serum testosterone concentrations did not show any significant changes after abamectin exposure. Abamectin administration was associated with decreased sperm count and motility and increased seminiferous tubule damage. In addition, significant elevations in the 4-hydroxy-2-nonenal (4-HNE)-modified proteins and poly(ADP-ribose) (PAR) expression, as markers for oxidative stress and poly(ADP-ribose) polymerase (PARP) activation, were observed in testes of rats exposed to abamectin. These results showed that abamectin exposure induces testicular damage and affects sperm dynamics. Oxidative stress-mediated PARP activation might be one of the possible mechanism(s) underlying testicular damage induced by abamectin.

1. Introduction

Abamectin (ABM) is a macrocyclic lactone product derived from the soil microorganism *Streptomyces avermitilis*. It is a mixture of avermectins containing about 80% avermectin B1a and 20% avermectin B1b (Burg et al., 1979; Fisher and Mrozik, 1989). These two components, B1a and B1b, have similar biological and toxicological properties (Lankas and Gordon, 1989). ABM is used as an insecticide and acaricide in many parts of the world. It inhibits the gamma-aminobutyric acid (GABA) induced neurotransmission, and causes paralysis in parasites (Campbell et al., 1983; Shoop et al., 1995; Turner and Schaeffer, 1989). ABM is also a chloride channel inhibitor, which makes it likely to affect the membrane stability (Korystov et al., 1999). ABM undergoes little metabolism within the target organism and most of the dose given to the animal is hence excreted as parent compound, primarily in the feces with less than 2% in urine (Gruber et al., 1990). They are highly lipophilic substances and dissolve in most organic solvents, but are poorly soluble in water (Roth et al., 1993). Although pesticides like ABM may be valuable in agriculture, it may be highly toxic to mammals (Moline et al., 2000).

In recent years, reproductive toxicity has been a topic of increasing interest and concern, as human exposure to a considerable number of potential toxicants is unavoidable due to contamination of air, water, ground, food, beverages, drugs and household items (Klinefelter et al., 2002; Pasqualotto et al., 2004). Environmental exposure to these agents may cause serious health risks including fertility and reproductive function. Previous reports have indicated a strong link between male infertility and exposure to more than 50 pesticides (Cox, 1996; Victor-Costa et al., 2010; Manfo et al., 2010; Tiwari et al., 2011). One of these pesticides, ABM, is used extensively all over the world. The widespread use of ABM has stimulated research into the possible existence of effects related with their reproductive toxic activity. Despite the large amounts of research on the various toxic effects of ABM (Hsu et al., 2001; Delgado and Paumgartten, 2004; Soyuncu et al., 2007; Sun et al., 2010), there are limited number of studies evaluating its effect on fertility and reproduction (Elbetieha and Da'as, 2003; Xu et al., 2005). Furthermore, the mechanism(s) underlying its possible harmful effects on male fertility is not known.

The presence of poly(ADP-ribose) polymerase (PARP), a DNA repair enzyme, has been detected in the nuclei of a variety of tissue types including testis (Di Meglio et al., 2004; Tramontano et al.,

* Corresponding author. Address: Department of Histology and Embryology, Faculty of Medicine, Akdeniz University, Antalya 07070, Turkey. Fax: +90 242 227 44 86.
E-mail address: cilerozenci@akdeniz.edu.tr (C. Celik-Ozenci).

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2005; Tekcan et al., 2011). It has recently been shown in male germ cells, specifically during stage VII of spermatogenesis (Agarwal et al., 2009). Agarwal et al. (2009) have described the possible biological significance of PARP in mammalian cells with the focus on male reproduction. Under physiological conditions or limited DNA damage, PARP plays a protective role (Spina-Purrello et al., 2008). On the other hand, after massive DNA damage following oxidative stress, PARP plays a crucial role in cell death program either by its over-activation or by participation to an extracellular signal regulated cascade. It has been reported that ABM decreases cell viability and SOD activity and increases lipid peroxidation of hepatocytes (El-Shenawy, 2010). As a consequence of increased oxidative stress due to ABM exposure, the overactivation of the PARP pathway in testis tissue may cause harmful effects on testes. Taking the above into account, the aim of the present study was to investigate whether there is a relationship between male reproductive function and exposure to ABM, a common pesticide being used by farmworkers. In the present study we also sought to elucidate the mechanism(s) underpinning the gonadal effects of exposure to ABM on male rats.

2. Materials and methods

2.1. Animals and abamectin exposure protocol

A total of 48 male Sprague–Dawley rats aged 14 weeks and weighing 300–400 g were used in this study. All procedures involving animals were performed in accordance with the guidelines of the University of Akdeniz, Faculty of Medicine, Animal Care and Use Committee with 06-05/01 approval number.

In the present study, the effects of ABM on reproductive system of male rats have been determined by exposing the animals to different doses (1 and 4 mg/kg/day) equivalent to 10% and 40%, respectively, of its oral LD_{50}, 10 mg/kg (Tomlin, 1994). Rats were given low [1 mg/kg/day, Low dose group (LDG) $n = 8$] and high [4 mg/kg/day, High dose group (HDG), $n = 8$] doses of ABM in sesame oil by oral gavage for 1 week (subacute effects) and for 6 weeks (subchronic effects). ABM (97.1% purity) was purchased from Sigma–Aldrich (PESTANAL®, analytical standard). Control animals ($n = 8$) received a similar volume of sesame oil vehicle. Rats were anesthetized with a cocktail of ketamine hydrochloride (90 mg/kg) and xylazine (10 mg/kg) administered intramuscularly (i.m.) before sacrifice of each rat and removal of the testes, epididymis and blood sampling. From each animal, two blood samples were collected to test tubes with EDTA (for plasma measurements) and without EDTA (for serum measurements).

2.2. Assessment of abamectin levels in plasma, testis, and epididiymis

2.2.1. Materials

Acetonitrile (ACN), methanol, triethylamine (TEA), *N*-methylimidazole (NMI) and trifluoroacetic anhydride (TFAA; analytical grade) were supplied from Merck (Darmstadt, Germany). ABM and doramectin (Sigma–Aldrich, analytical standard) were used as standard reference materials.

2.2.2. HPLC conditions

The HPLC system (Shimadzu) consisted of a LC10AD VP pump and a Shimadzu RF-10AXL fluorescence detector (excitation wavelength 365 nm; emission wavelength 470 nm). The separation was carried out on a Phenomenex Luna 3 μm C18(2) column (150 × 4.6 mm i.d.; 3 μm particle size) with a Phenomenex precolumn C18 (ODS, Octadecyl; 4.0 × 3.0 mm i.d.; 5 μm particle size). The mobile phase consisting of methanol–ACN–water (95 + 3 + 2, v/v/v) was pumped at 1.1 ml/min and 20 μl of sample was injected into the HPLC system.

2.2.3. Plasma sample preparation procedure

One milliliter of ACN:water (4 + 1, v/v) was added to each plasma sample (1 ml) and vortexed for 5 min. The samples were centrifuged at room temperature for 5 min at 2000*g*. After centrifugation, the supernatant was taken and transferred to a reservoir connected to a Bakerbond Octyl (C8) cartridge. The cartridge was previously activated with 5 ml of methanol and conditioned with 5 ml of water. After applying the sample extract, the cartridge was washed with 2 ml of water followed by 1 ml water:methanol (3 + 1, v/v). The analyte was eluted with 1.2 ml of methanol, collected in a polypropylene test tube and evaporated to dryness under nitrogen at 60 °C.

2.2.4. Tissue sample preparation procedure

Five ml of ACN was added to 1 mg weighted, homogenized tissues (testis and epididymis) and vortexed for 5 min. Samples were placed in ultrasonic shaker for 10 min. After centrifugation (10 min, 2600 rpm, room temperature), a 5 ml portion of extract was taken and diluted to 10 ml with doubly distilled water followed by addition of 50 μl of TEA. The sample was then transferred to a reservoir connected to a Bakerbond Octyl (C8) cartridge. The cartridge was previously activated with 5 mL of ACN and conditioned with 5 ml of ACN–water–TEA (50 + 50 + 0.1, v/v/v). After applying the sample extract, the analyte was eluted with 5 ml of ACN, collected in a polypropylene test tube and evaporated to dryness under nitrogen at 60 °C. Plasma and tissue samples were then derivatized with 100 μl of NMI in ACN (1 + 1, v/v) and 150 μl of TFAA in ACN (1 + 1, v/v). An 20 μl aliquot of the sample was then injected into the HPLC system.

For quantification purposes, calibration curves for ABM were prepared. The addition of 20 μl of the standard working solutions resulted in calibration curves with ABM concentrations of 1–100 ng/ml plasma. The curves were linear over this range ($r = 0.998$). The detection limit (LOD) and the limit of quantitation (LOQ) were defined as three and 10 standard deviation plus mean blank value, respectively. LOD and LOQ values were calculated as 0.05 and 0.12 ng/ml for ABM, respectively.

2.3. Histological evaluation

Left testicular tissues of rats were fixed in Bouin's fixative for 4 h, dehydrated in ethanol, embedded in paraffin, and stained with periodic acid Schiff (PAS) for histopathological evaluations. Tubule degeneration was evaluated in each group by Johnsen score (Johnsen, 1970). The Johnsen scoring system is principally based on the progressive degeneration of germinal epithelium and a successive loss of the most mature cell types during testicular damage. Johnsen's score was used to categorize the spermatogenesis. The scores from the control testes were compared with those of testes exposed to ABM. It applies a grade from 1 to 10 to each tubule cross section according to the following criteria: 10 = complete spermatogenesis and perfect tubules; 9 = many spermatozoa present and disorganized spermatogenesis; 8 = only a few spermatozoa present; 7 = no spermatozoa but many spermatids present; 6 = only a few spermatids present; 5 = no spermatozoa or spermatids but many spermatocytes present; 4 = only a few spermatocytes present; 3 = only spermatogonia present; 2 = no germ cells but only Sertoli cells present; 1 = no germ cells and no Sertoli cells present.

2.4. Immunohistochemistry

Sections were deparaffinized and blocked for endogenous peroxidase activity with methanol containing 3% H_2O_2 for 10 min and for nonspecific binding with Ultra V Block (Labvision,