Loss of the central rachis and synaptonemal complexes during meiotic prophase in female *Ascaris lumbricoides var. suum* after exposure to albendazole

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Abstract

Albendazole, a benzimidazole anthelmintic, interferes with the formation of microtubules and inhibits meiosis in the nematode *Ascaris lumbricoides var. suum*. Pigs treated with albendazole had worms in their uteri that had a severely deteriorated central rachis, complete loss of synaptonemal complexes and irregular oocytes at meiotic prophase I. The nuclear matrix and envelope were poorly formed and there was formation of accessory nuclei. This study represents the first examination of the changes in meiotic nuclear architecture and meiotic chromosomes after exposure to albendazole. These results provide the basis for the loss of fecundity in *A. suum* after exposure to albendazole resulting in control in the population of the parasitic nematode.

Introduction

Helminth infections of livestock and humans cause serious economic loss and disease throughout the world. With the introduction of the anthelmintic benzimidazoles, and other drugs, there has been substantial progress in chemotherapy of helminth infections (Kohler, 2001). Control of *Ascaris lumbricoides var. suum* (*A. suum*) parasites, using the benzimidazole anthelmintic albendazole, is mediated through the selective binding to nematode β-tubulins (Lacey, 1990). This results in inhibition of polymerization and prevents the formation of microtubules and cell division (Shrestha et al., 2016). Disruption of microtubules leads to loss of chromosome segregation and disruption of kinetochore function during meiosis resulting in aneuploidy, infertility and loss of fecundity (Dawson et al., 1984; Morrissette et al., 2004; Lyons-Abbott et al., 2010). The kinetochore is a specialized structure on the centromeric region of chromosomes comprising specific proteins which regulate the attachment of spindle microtubules during mitosis and meiosis. If the chromosomes are not segregated equally to the gametes during meiosis, there will be an unequal number of chromosomes (aneuploidy) in each gamete. Thus, the loss of the cytoplasmic microtubules results in the death of the nematodes (Shrestha et al., 2016) and interferes with other cellular functions including metabolism and cellular transport of proteins (Amini et al., 2014). Albendazole has also been used as anticancer and antiparasitic drugs (Jordan & Kamath, 2007; Sant’anna et al., 2013).

In this study, the animal parasitic nematode *Ascaris lumbricoides var. suum* was exposed to albendazole through treatment of its host animal, *Sus domesticus*. It reproduces primarily via sexual reproduction (amphimixis). The two sexes are separate and there is distinct sexual dimorphism between the male and female. The adult female has a pair of ovaries and 12 pairs of autosomes with zero X chromosomes (2n = 24A). Nuclei during the pachytene stage are arranged peripherally around a central rachis which provides for synchronous development in that region of the gonad. Pachytene is a specific stage of the first meiotic prophase in which the homologous pairs of chromosomes undergo the process of crossing-over and recombination. The adult male has a single testis and 12 pairs of autosomes in each spermatocyte nucleus (thus, n = 12 for both the male and female). However, the male does have five univalent sex chromosomes, designated ‘Y’ to infer that they are not present in females (Goldstein & Moens, 1976). These five Y chromosomes are linked together and distributed to the spermatocyte, which is destined to produce a male. Spermatocytes without the univalent Y chromosomes would produce a female. Thus, dimorphism of spermatozoa is associated with dimorphism of sex. The two sexes experience an unequal number of sex chromosomes, similar to humans. They must compensate for this state of aneuploidy and develop mechanisms for gene expression and dosage compensation.

In *A. suum* the telogenic gonad contains gametogonia which originate from the proximal end of the gonad and move towards the distal end. As they migrate, they undergo successive stages of gametogenesis and are arranged in a honeycomb pattern. It is only at pachytene that there is complete synchrony of the oocytes, since they are arranged peripherally around a
central rachis and are in communication of each via cytoplasmic bridges (Amini et al., 2014). Each of these oocytes are in prophase I of meiosis and contains synaptonemal complexes (SCs), which are tripartite, proteinaceous structures that are found between homologous paired chromosomes at pachytene (Goldstein, 1981a, b). The SC comprises two lateral elements, which are the axial cores of the homologues and a proteinaceous central element. The SC has been highly conserved throughout evolution and occurs in virtually all organisms that reproduce via meiosis. Its role is twofold: (1) maintenance of proximity of homologous chromosomal segments, such that the axial cores of the chromosome become the lateral elements of the SC; and (2) regulation of ordered meiotic disjunction, in which case the SC is maintained in the chiasma. Irregular chromosome segregation and nondisjunction results in the formation of non-viable gametes, aneuploidy and loss of fecundity.

This study represents the first examination of the changes in meiotic nuclear architecture and meiotic chromosomes after exposure to albendazole and provides the basis for the anthelmintic control of nematodes. In this paper, changes in meiotic nuclei, loss of the central rachis, formation of accessory nuclei and loss of SCs in albendazole-exposed nematodes are described.

Materials and methods

*Ascaris suum* worms were collected from 20 humanely slaughtered pigs as per Food Safety and Inspection Service guidelines (9 C.F.R. 313.1–313.90) (*Sus domesticus*), near El Paso, TX. Ten of these pigs had been treated *per os* with the recommended single dosage of 5 mg/kg albendazole. Ten pigs were selected that had not been treated with any anthelmintic. One female worm was obtained from the intestine of each of the treated and non-treated pigs and processed for electron microscopy.

For electron microscopy, the worms were placed into a phosphate-buffered 2% glutaraldehyde solution, pH 7.2, and the ovaries were immediately removed, transferred to fresh fixative and kept in the refrigerator overnight. Post fixation was in Dalton’s osmium–chromic acid (Zickler & Olson, 1975) for 2 h at room temperature, followed by dehydration through an alcohol and propylene oxide series, embedded in Epon and stained with uranyl acetate and Fiske lead citrate (Fiske, 1966). Ultrathin sections were cut on a Porter–Blum ultramicrotome and examined with a Zeiss electron microscope. Light microscopy was performed using the technique of Goldstein & Braselton (1975), which is a rapid whole-mount procedure using a single-solution Hoyer’s mounting medium haematoxylin stain. This facilitates light microscopic examination of nuclear events.

Ten worms were randomly selected from each of the groups: (1) control-unexposed and (2) exposed. In each group, the following parameters were assessed: (1) presence of the SC; (2) presence of a normal bipartite nuclear envelope that was completely contiguous with the nucleoplasm; and (3) presence of the central rachis in the germinal zone at the stage of meiosis prophase I. All statistical analysis in this study were performed using the open-source RStudio software (Version 1.2.5033, 2019 RStudio, Inc.).

Results

The ultrastructure of oocytes in meiotic prophase I revealed numerous aberrations after exposure to albendazole. In untreated worms, the oocytes at meiotic prophase I were arranged peripherally around a central rachis (fig. 1). The epidermal cells of the ovary appeared normal with a contiguous cell membrane and all organelles. The double membrane of the nuclear envelope of each oocyte was completely contiguous with the nucleoplasm. In worms exposed to albendazole, the central rachis deteriorated and SCs were not present in the oocyte nuclei. Remnants of dissociated SCs were discarded into the central rachis (fig. 2). There were no secretory vesicles or vacuoles containing dissociated SCs.

Fig. 1. Meiotic prophase cross-section through the anterior portion of the ovary of an untreated female of *Ascaris lumbricoides* var. *suum*. Oocytes (O) surround the central rachis (R). Epithelial cell (E) of the ovarian wall (>2000).

Fig. 2. The branching rachis (R) contains remnants of dissociated synaptonemal complexes (arrowheads) following treatment with albendazole (>4900).
two proportions were performed to compare the probability of each of the three outcomes between the exposure/control populations. For each of the three proportion tests, the resulting (identical) chi-squared (with Yates’ correction for continuity) values (16.2) and $P$-values (0.00000285) suggested that the null hypothesis of no difference in propensities could be rejected in favour of the alternative hypothesis of increased propensity in the treated population at both per comparison and family-wise significance level $\alpha = 0.05$. Since the data were collected in a randomized controlled setting, a causal effect behind the treatment can be further inferred.

**Discussion**

Exposure to albendazole results in abnormal chromosome segregation during meiosis. Meiosis is a specialized cell division that halves the chromosome number and results in the production of gametes (Goldstein, 1981a, b). Meiosis normally produces gametes containing exactly one copy of each chromosome. Meiotic errors lead to gametes with incorrect chromosome numbers, a major cause of infertility and decreased fecundity (Mikwar et al., 2020). A key step in meiosis I is the separation of homologous chromosomes, which is mediated by the SC. Homologous chromosomes first become physically linked by recombination, which keeps them together until they attach properly at their centromeres via microtubules to the apparatus that will pull them to opposite sides of the cell (Gladston et al., 2009). Disruption of microtubules leads to the production of aneuploidy nuclei and non-viable zygotes (Mikwar et al., 2020).

In *A. suum* the central rachis provides the infrastructure for the peripherally arranged syncytial oocytes (Prestage, 1960). Each oocyte is connected via a cytoplasmic bridge to the central rachis. Within a restricted zone, all the oocytes are in meiotic prophase I and normally contain SCs between the homologous paired chromosomes. Recombination and disjunction of the chromosomes follow with equal distribution into developing oocytes. Loss of the central rachis, as observed in albendazole-exposed *A. suum* oocytes resulted in complete loss of the syncytium and synchrony of development. Loss of the SC results in the production of aneuploidy gametes (Goldstein, 1981a, b).

Accessory nuclei found in the *A. suum* oocyte nuclei contained chromosomal material as evidenced by the positive stain reaction for nucleic acids by either acridine orange or by the procedure of Goldstein & Braselton (1975). The formation of accessory nuclei in *A. suum* was present only after exposure to albendazole. Their function is not clear, but they most likely represent premature senescence, as benzimidazoles are known to induce senescence. The accessory nuclei are eliminated starting with the fourth embryonic division through the process of chromatin diminution (Goldstein, 1981a, b).

**Albendazole**

Albendazole binds specifically to $\beta$-tubulin, which is a protein subunit of the microtubules that has a fundamental role in segregation of chromosomes during meiosis. Albendazole-specific binding sites on $\beta$-tubulin lead to local disruption of the protein resulting in inhibition of the formation of microtubules (Horton, 2000; Kohler, 2001). The anthelmintic efficacy is due to the ability of albendazole to compromise the cytoskeleton through this selective unfolding of a small region within the $\beta$-tubulin monomer (Kohler, 2001). The effects of albendazoles on *A. suum*, and other nematodes, such as *Litomosoides* (Cardenas et al., 2010) and

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**Fig. 3.** The synaptonemal complex from an untreated female *Ascaris lumbricoides* var. *suum* oocyte is a tripartite structure. The lateral element (LE) central element is the line between the two lateral elements. CH, chromatin; C, centriole ($\times$80,000).

**Fig. 4.** Accessory nuclei (AN, arrow) in albendazole-treated oocytes of *Ascaris lumbricoides* var. *suum*. ON, oocyte nucleus; CH, chromatin; G, granular structures ($\times$40,000).
*Caenorhabditis elegans*, (Sant’anna et al., 2013) include impaired locomotion, impaired microtubule formation leading to cellular disintegration affecting the germlinal zone of the ovary and the oocytes. Since microtubules are involved in so many critical aspects of the cell and reproduction, the albendazole-induced destruction eventually leads to the death of the organism (Kohler, 2001). The sensitivity of the nematode *C. elegans* to albendazole is mediated by a single gene, *ben-1*, which encodes β-tubulin. This has provided a platform to investigate the molecular basis of albendazole resistance in parasitic nematodes (Holden-Dye & Walker, 2007). Studies are in process to determine if *A. suum* has the same genetic sequence.

In this paper, the effectiveness of albendazole to reduce fecundity in *A. suum* is demonstrated by the changes in the oocytes and the rachis. The loss of SCs at meiotic prophase 1 is significant because viable oocytes cannot be formed. The result is the limitation of parasitic infections in individual livestock and human populations thereby controlling transmission (CDC, 2019).

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### References

Amini R, et al. (2014) *C. elegans* anillin proteins regulate intercellular bridge stability and germline syncytial organization. *Journal of Cell Biology* 206, 129–143.

Cardenas M, et al. (2010) Effects of albendazole on *Litomosoides carinii* (Nematoda:Filaroidea) females in vivo. *Parasitology Research* 107, 817–826.

Center for Disease Control (www.cdc.gov/parasites/ascariasis/biology.html), 2019

Dawson P, et al. (1984) A comparison of the interaction of anthelmintic benzimidazoles with tubulin isolated from mammalian tissue and the parasitic nematode *Ascaridia galli*. *Biochemical Pharmacology* 33(7), 1069–1074.

Fiske S (1966) An adaptation of Reynold’s lead citrate stain for high resolution autoradiography. *Journal of Microscopy* 6, 355–360.

Gladston D, et al. (2009) The synaptonemal Complex protein Zp3 promotes Bi-orientation of centromeres at meiosis. *PLOS Genetics* 5(12), 771–781.

Goldstein P (1981a) Accessory nuclei in female in *Ascaris suum*. *Journal of Parasitology* 65, 697–701.

Goldstein P (1981b) Sex determination in nematodes. pp. 37–60 in Zuckerman B, R Rhode (Eds) *Plant-Parasitic Nematodes*, Vol. 3, NY, Academic Press.

Goldstein P and Braselton J (1975) A rapid whole-mount staining procedure for *Ascaris* embryo chromosomes. *Stain Technology* 50, 362–363.

Goldstein P and Moens P (1976) Karyotype analysis of *Ascaris* male and female pachytene nuclei by 3-D reconstruction from electron microscopy of serial sections. *Chromosoma* 58, 101–111.

Holden-Dye, L. & Walker, R.J. (2007) Anthelmintic drugs, *WormBook*, ed. The *C. elegans* Research Community. *WormBook*, doi/10.1895/wormbook.1.143.1, http://www.wormbook.org.

Horton J (2000) Albendazole: A review of anthelmintic efficacy and safety in humans. *Parasitology* 121, 113–132.

Jordan L and Kamath P (2007) How do microtubule-targeted drugs work? An overview. *Current Cancer Drug Targets* 7, 730–742.

Kohler P (2001) The biochemical basis of anthelmintic action and resistance. *International Journal for Parasitology* 31, 336–345.

Lacey E (1990) Mode of action of benzimidazoles. *Parasitology Today* 6, 112–115.

Lyons-Abbott DL, et al. (2010) α-Tubulin mutations alter oryzalin affinity and microtubule assembly properties to confer dinitroaniline resistance. *Eukaryotic Cell* 9(12), 1825–1834.

Mikwar A, et al. (2020) Mechanisms of oocyte aneuploidy associates with advanced maternal age. *Mutation Research* 785, 1–20.

Morrisette A, et al. (2004) Dinitroanilines bind alpha-tubulin to disrupt microtubules. *Molecular Biology of the Cell* 15(4), 1960–1968.

Prestage J (1960) The fine structure of the growth region of ovary in *Ascaris lumbricoides* var. *Suum* with special reference to the rachis. *Journal of Parasitology* 46, 69–78.

Sant’anna V, et al. (2013) Caenorhabditis elegans as a model for the screening of anthelmintic compounds: Ultrastructural study of the effects of albendazole. *Experimental Parasitology* 135, 1–8.

Shrestha V, et al. (2016) Comparison of anthelmintic activity between bisaryl benzyl benzyl piperazine and benzimidazole through a selective interaction with β-tubulin. *International Journal of Pharmaceutical Sciences Research* 7, 1547–1555.

Zickler D and Olson L (1975) The synaptonemal complex and the spindle plaque during meiosis in yeast. *Chromosoma* 50, 1–23.

| Table 1. *Ascaris suum* female worms exposed to albendazole resulted in complete loss of the synaptonemal complexes (SCs), rachis and a properly formed nuclear envelope (NE) around the developing oocyte. |
| A | B | C |
|---|---|---|
| SCs present | Contiguous NE | Rachis present at pachytene |
| Yes | No | Yes | No | Yes | No |
| Control | 10 | 0 | 10 | 0 | 10 | 0 |
| Exposed | 0 | 10 | 0 | 10 | 0 | 10 |

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