Fate of Microfilariae of Dirofilaria Immitis Following Use of Levamisole as a Microfilaricide

C.F. Simpson and R.F. Jackson
College of Veterinary Medicine, Box J-136 JHMHC, University of Florida, Gainesville, FL 32610, USA

Abstract. A pretreatment liver biopsy was secured from each of three dogs with Dirofilaria immitis microfilariae counts of 29,500, 24,700, and 76,700/ml blood, respectively. Post-treatment biopsies were obtained 30 h later following treatment with a single dose of levamisole and a reduction in microfilariae counts of up to 80%. Both pre- and post-treatment biopsies were examined by light and electron microscopy. Microfilariae in dilated sinusoids of pretreatment liver biopsies were not degenerated and were unattended by an inflammatory reaction when examined by either method. However, degenerated microfilariae were present within granulomas in post-treatment liver biopsies examined by light and electron microscopy. Several stages of degeneration of microfilariae, including phagocytosis, were identified by the latter method.

Introduction

The gross and microscopic lesions common in Dirofilaria immitis infection of dogs are associated with the presence of adult worms. It is now accepted that microfilariae of D. immitis do not induce microscopic lesions other than membranous glomerulonephritis in dogs with high microfilariae counts (Simpson et al. 1974; Casey and Splitter 1975). Levamisole has been used as a microfilaricidal agent in heartworm infection of dogs; the drug reduces microfilariae counts dramatically after 6–10 days of treatment (Bradley 1976; Jackson 1977; Mills and Amis 1975). To date, it has not been reported that a tissue response results from the destruction of microfilariae of D. immitis following the use of levamisole or other microfilaricidal drugs. To obtain an answer to this question, we examined the livers of heavily infected dogs that were treated with levamisole. The results of this investigation are the subject of the present paper.

Offprint requests to: Ch. F. Simpson
Materials and Methods

Three dogs with spontaneous infections with heartworms were studied. Each dog was administered a single, oral dose of levamisole (11.0 mg/kg) 24 h after obtaining blood for microfilariae counts and pretreatment liver biopsies (Osborne 1971). Blood for microfilariae counts was obtained again 24 h after treatment, and the animals were killed and post-treatment liver samples were obtained 30 h after treatment. As a result, each dog served as its own control.

Liver biopsies were examined by light and electron microscopy. For light microscopy, specimens were fixed in 10% neutral formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin-eosin. For electron microscopy, tissue was fixed in 3.5% buffered glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in Araldite. Thin sections on grids, stained with uranyl acetate and lead citrate, were examined in a Philips EM200 electron microscope.

Results

Before treatment, microfilariae counts of the three dogs were 29,500, 24,700, and 76,700/ml blood. Microfilariae counts were reduced in the three dogs from pretreatment counts by 43%, 80%, and 70%, respectively, 24 h following the single, oral dose of levamisole.

In pretreatment liver biopsies examined by light microscopy, microfilariae were present in dilated sinusoids without evidence of an inflammatory response. In post-treatment liver biopsies examined by light microscopy, there were multifocal, oval to round granulomatous nodules, 35 to 53 μm in diameter (Fig. 1), in the sinusoids. They contained degenerated microfilariae surrounded by reticuloendothelial cells and an occasional neutrophil (Fig. 2).

Microfilariae were present in the dilated sinusoids of pretreatment liver biopsies examined by electron microscopy; they were not accompanied by inflammatory cells. The microfilariae were confined by a thick, close-fitting, annulated cuticle (Fig. 3). Beneath the cuticle were muscle cells with filaments, mitochondria, nuclei, and glycogen granules (Fig. 4).

Degenerated microfilariae were present in post-treatment liver biopsies examined by electron microscopy. They were located within granulomas in the sinusoids, either free or phagocyted by macrophages of the granuloma. In the earliest stage of degeneration, pseudopods of macrophages surrounded a microfilaria without engulfing it. Such nonphagocyted microfilariae contained vasculolated nuclei, and there was subcuticular necrosis (Fig. 5). In the intermediate stage of degeneration, microfilariae, although not phagocyted, were vacuolated, with their cuticle slightly separated from the body (Fig. 6). More advanced stages of degeneration of the parasites followed phagocytosis, the degenerative changes including karyorrhexis, cytoplasmic necrosis, cytoplasmic vacuolization, and extensive separation of the distorted cuticle from the body (Figs. 7, 8). As a result, a microfilaria was very vacuolated and contained only remnants of nuclei. Ultimately, all that remained of phagocyted parasites was a fragmented, collapsed cuticle (Figs. 9, 10).