ORIGINAL RESEARCH

The Use of Ivermectin to Kill *Ixodes Scapularis* Ticks Feeding on Humans

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Objective.—The purpose of this study was to determine whether 400 µg/kg oral ivermectin is able to kill *Ixodes scapularis* nymphs and adult female ticks feeding on humans.

Methods.—Ten study subjects each wore 2 ostomy bags, the one containing 24 *I scapularis* nymphs, and the other containing 24 *I scapularis* adult females. Twenty-four hours after the ostomy bags were attached, study subjects received either 400 µg/kg ivermectin or placebo. Thirty hours after the ivermectin or placebo was consumed, the ticks were removed, and mortality determined in a double-blinded manner.

Results.—Eleven percent of the *I scapularis* nymphs attached in the ivermectin group compared with 17% in the placebo. Mortality for the *I scapularis* nymphs that attached at the time of removal was 55% in the ivermectin group and 47% in the placebo group. Mortality for the *I scapularis* nymphs 5 days after removal was 92% in the ivermectin group and 88% for the placebo. Three percent of the *I scapularis* adults attached in the ivermectin group compared with 9% in the placebo group. Mortality for *I scapularis* adults was 0% on day 3 and 33% on day 8 for both the ivermectin and placebo groups. There were statistically insignificant differences in the mortality rates between *I scapularis* nymphs and adults exposed to ivermectin or placebo.

Conclusions.—There were a high number of ticks that died in both groups but the data do not support our hypothesis that ivermectin can kill *I scapularis*. The study was not designed to determine whether it could prevent the transmission of tick-borne illness.

Key words: ivermectin, tick, *Ixodes scapularis*, tick-borne disease, Lyme disease, prevention

Introduction

The deer tick, or blacklegged tick, *Ixodes scapularis*, is the most important vector of tick-borne diseases to humans in the United States.^{1–5} *Ixodes scapularis* can transmit *Borrelia burgdorferi*, *Borrelia miyamotoi*, *Ehrlichia* sp, *Theileria microti*, *Anaplasma phagocytophilum*, and Powassan encephalitis virus which cause Lyme disease, human ehrlichiosis, human babesiosis, human

granulocytic anaplasmosis, and human tick-borne encephalitis, respectively.^{1–5}

Lyme disease is responsible for more than 90% of all human vector-borne disease in the United States, with an estimated 35,000 cases a year and an additional 60,000 cases annually in Europe.^{1,2} Lyme disease is most common in the northeastern and midwestern United States where *I scapularis* is the principal vector.^{1,2} In Connecticut, approximately 20% of *I scapularis* nymphs and 30% to 60% of *I scapularis* adults are infected with *B burgdorferi*.³ The *I scapularis* nymphs are responsible for approximately 80% of human cases of Lyme diseases, but the adults are also capable of transmitting the infection. Lyme disease can be difficult to diagnose clinically, and untreated infections can result in neurologic, cardiac, and rheumatologic sequelae.^{1,2,4–6} Unfortunately, 30% to 50% of people infected with *B*

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burgdorferi report no history of a tick bite and 20% to 30% of people do not produce the characteristic erythema migrans rash.^{1,2,4-6} Most cases of Lyme disease are acquired in activities around the home, for example, pets may bring *I scapularis* into the home, making it possible to acquire Lyme disease without venturing outdoors.⁶

The *I scapularis* tick has a 2-year, 4-stage lifecycle including egg, larva, nymph, and adult. Each life stage feeds once for 3 to 5 days. Larvae and nymphs most commonly become infected with *B burgdorferi* when they feed on infected white-footed mice (*Peromyscus leucopus*). For *I scapularis* to transmit *B burgdorferi* to humans, the bacteria must first leave the midgut and migrate to the salivary glands, a process requiring a minimum of 36 to 48 hours.^{1,2,4,5}

Current recommendations for preventing tick-borne disease are often impractical and have limited effectiveness. They focus on reducing deer and mice populations, avoiding tick habitat, wearing light-colored clothing to aid in identifying the ticks, tucking socks into your pants, and applying insect repellents and insecticides.⁷ The US Centers for Disease Control and Prevention (CDC) recommends using insect repellents containing 30% to 50% N,N-diethylmetatoluamide (DEET) or picaridin, but both require frequent reapplication and are only effective on areas of the body and clothing where they are applied.⁷ Ticks may walk across a surface containing DEET to attach to skin where DEET is absent.³ Permethrin acts more as an insecticide than a repellent and will kill ticks after prolonged exposure. Permethrin is applied to clothing and is not indicated for application on skin.³ Prophylaxis after exposure with 200 mg oral doxycycline within 72 hours of tick attachment has been shown to reduce the risk of infection from *B burgdorferi*.⁵

Ivermectin is one of the most ubiquitous antiparasitic drugs, with more than 5 billion doses of ivermectin-containing products sold worldwide.⁸ A single 150 to 200 µg/kg dose of ivermectin has shown effectiveness against several human parasites, including the ectoparasites lice, scabies, mosquitoes, and bed bugs.⁹⁻¹² Ivermectin has been used extensively in the control of Onchocerciasis (river blindness) and filariasis and is administered to an estimated 50 million people annually.^{13,14} As the only approved endectocide for human use, approximately 1 billion ivermectin treatments have been given to humans over the past 30 years.^{10,13}

Ivermectin acts on the glutamate-gated chloride channel, which is lacking in humans, and to a lesser extent the γ-aminobutyric acid (GABA)-gated chloride channel, causing hyperpolarization of invertebrate nerve and muscle cells.^{8,14} In humans, ivermectin is widely distributed in the body, with a peak plasma level occurring

approximately 4 hours after taking the drug.^{14,15} The peak concentration of ivermectin in sebum, sweat, and squames is approximately 8 hours after drug consumption, and levels drop after 24 hours.¹⁵ The antiparasitic effects of ivermectin appear to persist longer than the 18 to 22 hour half-life.^{14,15}

Although ivermectin has been used in veterinary medicine to kill ticks on animals, there is only a single previous report about the use of ivermectin to kill ticks on humans.¹¹ Our study was designed to determine whether a single dose of oral ivermectin could be used to kill *I scapularis* nymphs and adult females attached to the body of human hosts. We reasoned that if ivermectin could kill *I scapularis* within 30 hours of tick attachment, then *B burgdorferi* could not be transmitted and ivermectin potentially could be used to prevent tick-borne disease. Hypothetically, if ivermectin readily killed attached and feeding *I scapularis*, then it might prove to be more useful in preventing tick-borne diseases among certain groups of people (eg, loggers, soldiers, outdoor enthusiasts) than currently recommended methods.

Previously it has been shown that ivermectin is effective at killing several different human parasites at 200 µg/kg, but 400 mg/kg was shown to be more efficacious for lice with few additional side effects.⁸⁻¹⁵ We chose to use 400 µg/kg in our study with the rationale that a single large dose of the drug would be the ideal option should ivermectin ever be adopted for wider use in the prevention of tick-borne disease. Hypothetically, ivermectin could be taken shortly after a person has a potential tick exposure and would kill the tick when it starts to feed but before transmitting tick-borne disease. Because ivermectin has such a long half-life, it even could potentially be effective if the tick wandered on the body for several days before attaching and consuming a lethal dose of ivermectin.

The current research is a follow-up study to one conducted in 2011 suggesting that ivermectin might be able to kill ticks. However, too few ticks attached to study subjects to reach statistically meaningful conclusions.¹¹ In this study, we increased the number of study subjects, ticks, and attachment time to increase the number of attached ticks.¹¹

Methods

This study was approved by the Eastern Virginia Medical School and Old Dominion University Institutional Review Boards. The US Food and Drug Administration granted investigational new drug approval for the use of 400 µg/kg of ivermectin against *I scapularis* ticks in humans. In April 2013, 7 men and 3 women between the ages of 19 and 33 years participated in the

Figure 1. Modified syringe for injecting ticks into the ostomy bags. At the time of injection, the rubber washer and cheesecloth at the end of the syringe is removed and the plunger is depressed. Image courtesy of Dr Michael Levin.

study. All study subjects met inclusion and exclusion criteria. None of the study subjects was taking daily medications, or was pregnant or breastfeeding. None of the study subjects had ever had a seizure, asthma attack, hepatitis, or edema. Study subjects were followed up for potential adverse events from the time they signed the consent through the next 8 days. Physical examinations were performed at baseline on study days 1, 2, and 3. On study day 8, all study subjects received a final e-mail and were asked to report any new symptoms or change in previously reported complaints. All study subjects completed the study and received \$200 in compensation.

The study was a randomized, double-blind, placebo-controlled, phase 2 clinical trial to determine whether a single oral dose of 400 µg/kg ivermectin could kill feeding *I. scapularis* ticks on humans. Pathogen-free *I. scapularis* nymphs and adult female ticks were provided by Dr Michael Levin (Medical Entomology Laboratory, Rickettsial Zoonoses Branch, US Centers for Disease Control and Prevention, Atlanta, Georgia). The ticks were originally collected in Bridgeport, Connecticut, in 1999 and maintained for more than 20 generations by feeding all life stages upon tick- and pathogen-naïve New Zealand white rabbits. We received 10 modified syringes containing 24 *I. scapularis* nymphs and 10 modified syringes containing 24 *I. scapularis* adult females (Figure 1). The ticks were stored at 37°C and 90% humidity before being used in the study. The modified syringes had the distal end cut off and replaced by cheesecloth held in place by a rubber washer. The syringe plunger was also covered with cheesecloth. The adult female *I. scapularis* was used because it is more likely to feed than the adult male *I. scapularis*, which normally mate with the females without attaching to host skin.

Each study subject had two Securi-T (Genairex, Largo, FL) 6-inch, one-piece ostomy system bags cut to 2-3/8 inch internal diameter applied to their abdomen using Stomahesive paste (ConvaTec, Skillman, NJ). Osto-Bond (M.O.C., Vaudreuil, Quebec, Canada) skin bonding latex adhesive was then applied around the perimeter of the ostomy bag where it attached to the skin. Some study subjects also applied tape around the perimeter of the ostomy bag to assist in securing the bag to the abdomen (Figure 2). A syringe containing the ticks was then injected into each ostomy bag before it was manually sealed. Nine of 10 study subjects received super glue (Loctite, Rocky Hill, CT) to seal the ostomy bag closed. All study subjects also used tape or a manual locking closure device or both for the ostomy bag. Each study subject received 24 nymph and 24 adult female *I. scapularis* ticks in 2 separate ostomy bags. The modified syringes allowed for easy injection of the ticks inside the ostomy bags.

Study subjects had the ostomy bags secured to their abdomen and then the ticks placed inside on day 1 of the experiment. Twenty-four hours later (day 2) they returned to the laboratory and were randomly allocated to receive either 400 µg/kg ivermectin or placebo (empty gel capsule). There were 5 subjects in each arm.

Figure 2. Ostomy bag with *Ixodes scapularis* adult females.

Randomization to study intervention was determined by a random number generator, except for 1 study subject who was unable to return to the laboratory on day 2 because of an emergency unrelated to study participation. This subject was placed in the placebo group. On day 3, study subjects returned 54 hours after the ticks were initially placed (30 hours after ivermectin or placebo was ingested) to have all ticks removed. Dr Sonenshine, one of the study coauthors who is a tick expert with more than 30 years of tick research experience, removed, counted, and divided the ticks into the following four categories: alive attached, dead attached, alive unattached, and dead unattached. At the time of ostomy bag removal, all ticks were placed into test tubes, and after 5 days (day 8) mortality rates were again recorded by Dr Sonenshine.

Results

One hundred twenty *I scapularis* nymphs and adult females were exposed to subjects who received 400 µg/kg ivermectin; and an equal number were exposed to subjects who did not receive ivermectin (placebo). Eleven percent of the *I scapularis* nymphs attached in the ivermectin group compared with 17% in the placebo. Three percent of the *I scapularis* adults attached in the ivermectin group compared with 9% in the placebo. The data are summarized in the [Table 1](#).

On day 3, 54 hours after the ticks were placed on study subjects, and 30 hours after potential ivermectin exposure, 6 of 11 *I scapularis* nymphs (55%) that attached in the ivermectin group were dead compared with 9 of 19 (47%) nymphs (47%) that attached in the placebo group. On day 8, after 5 days of observation in a test tube, mortality of the *I scapularis* nymphs was 12 of 13 (92%) in the ivermectin group and 14 of 16 (88%) in the placebo group for ticks that attached. The mortality rates for *I scapularis* nymphs on day 3 and day 8 comparing the ivermectin and placebo groups did not reach statistical significance. Of the *I scapularis* nymphs

that did not attach or feed during the experiment, 77% were dead on day 8 in the ivermectin group and 71% in the placebo group.

On day 3, 0% of the *I scapularis* adults that attached in both the ivermectin and placebo groups were dead. On day 8, 33% of the *I scapularis* adults that attached in both the ivermectin and placebo groups were dead. On day 8, 73 of 114 of the *I scapularis* adults (64%) in the ivermectin group that did not attach or feed were dead compared with 42 of 96 (44%) in the placebo group.

Thirty-eight percent of the *I scapularis* nymphs in the ivermectin group were unaccounted for at the end of the experiment (ie, escaped from the ostomy bag) compared with 40% in the placebo group. One percent of the *I scapularis* adults in the ivermectin group were unaccounted for at the end of the experiment compared with 7% in the placebo group. The larger number of nymphs that escaped compared to adults is likely a reflection of their smaller size.

Nine of 10 study subjects received Loctite super glue. The single study subject who did not receive the super glue received ivermectin and had, on day 3, 5 alive unattached nymphs, 1 dead attached nymph, and 14 dead unattached nymphs as well as 2 alive attached adults and 21 alive unattached adults.

There are discrepancies between the total number of ticks that attached during the experiment as recorded on day 3 and day 8 even though all ticks were removed from study subjects on day 3. Only the ticks that were physically attached and feeding on day 3 when they were removed from the study subject's skin were counted as attached on day 3. On day 8, any tick that showed signs of having been attached and fed were counted as having been attached. A tick will usually not stop feeding and detach.

Study subjects were followed up for potential adverse events from the time of consent through the next 8 days. Symptoms developed in 4 study subjects during study participation. Three study subjects had itching, burning, or erythema within minutes of applying the Osto-Bond and ostomy bags and before the ticks were placed inside

Table 1. Data summary^a

	Mortality rates			
	Ticks that attached day 3	Ticks that attached day 8	Ticks that never attached day 8	Unaccounted for ticks day 8 (escaped)
<i>Ixodes scapularis</i> nymphs, ivermectin	6/11 (55%)	12/13 (92%)	48/62 (77%)	46/120 (38%)
<i>I scapularis</i> nymphs, placebo	9/19 (47%)	14/16 (88%)	30/42 (71%)	38/96 (40%)
<i>I scapularis</i> adult female, ivermectin	0/3 (0%)	1/3 (33%)	73/114 (64%)	3/120 (1%)
<i>I scapularis</i> adult female, placebo	0/7 (0%)	3/9 (33%)	42/96 (44%)	7/96 (7%)

^a Each group started with 120 ticks, but 1 study subject in the placebo group did not have ticks observed past day 3; all were reportedly dead.

the bag. One study subject required the ostomy bags to be removed but requested they be reattached without Osto-Bond, and they were taped to the abdomen using athletic tape. Two of these subjects took over-the-counter diphenhydramine that relieved their symptoms. One study subject reported nausea and another dizziness before either received ivermectin or placebo. In the ivermectin arm, 1 study subject complained of chest pain on day 6 and “stomach trouble” on day 7; both complaints resolved spontaneously without medical intervention. Headache developed in 1 subject in each group and resolved without medical intervention. One study subject was unable to return to the laboratory on study day 2 owing to involvement in a minor motor vehicle accident while driving to the study site. To maintain even study arm assignment, this subject was placed in the placebo group and never received any study drug. This study subject received a large amount of Loctite glue to close the ostomy bag and had reported previously that all her ticks died several hours after they were placed inside the ostomy bag and that none of the ticks attached to feed. The ticks from this study subject were discarded on day 3 and not observed on day 8.

Discussion

Tick mortality in both our placebo and ivermectin groups was so high that definitive conclusions about the effects of ivermectin on *I scapularis* should be reserved until the methodologic problems highlighted in our study are resolved. We would expect to see essentially a 0% mortality rate for ticks in the placebo group but our mortality rate was much higher.

Given the large number of ticks that died during the study, some shortly after they were placed in the ostomy

bag, we strongly suspect a toxic exposure. The temperature or humidity from inside the ostomy bag would not have killed the ticks. Ticks are very resistant to mechanical trauma, so that is an unlikely cause of their death. Ticks are able to survive in a sealed container for several days without ill effects, so hypoxia would not have contributed to mortality. Lastly, it is unlikely that the ticks died of an infectious process as the ticks were grossly normal before being put into the ostomy bags.

Our study did not look at the ability of ivermectin to prevent *I scapularis* from transmitting tick-borne pathogens. The transmission of *B burgdorferi* is an active process whereby *I scapularis* injects the bacteria in its saliva as it feeds. Ivermectin has been shown to inhibit tick somatic and pharyngeal muscles, so it may be possible that ticks briefly exposed to ivermectin will not transmit *B burgdorferi* even if the ticks do not die. However, our primary outcome was tick mortality because ticks that are dead will not transmit *B burgdorferi*, tick mortality can be readily observed, and infected ticks cannot be ethically applied to humans.

There are few published reports or clinical trials in the scientific literature involving the attachment of ticks to humans. This is the second known attempt at using an ostomy bag attached to the abdomen to secure the ticks to the study subject. Problems with ticks escaping from the bag, as well as premature tick death, mean that other methods of confining ticks to humans need to be developed. Methods of confining ticks to animals have been developed and include gluing (veterinary approved cyanoacrylate-based glues) plastic containers or stockinettes to the animal's skin, and then when the glue is dry, placing the ticks inside (Figure 3). However, these approaches are less practical for humans. We suspect that the ethyl cyanoacrylate-based glue contributed to our overall tick mortality and would recommend

Figure 3. A stockinette glued to a rabbit and tied at the top (left). Untying the stocking allows for direct visualization of the feeding ticks (right). Image courtesy of Dr Michael Levin.

avoiding acute exposure of the ticks to the glue vapor in future experiments. In addition, 1 or possibly even 2 additional days should be added to the tick exposure period before beginning the ivermectin treatment. This would insure a large population of attached feeding ticks at the time of drug administration.

STUDY LIMITATIONS

We had unacceptably high mortality in both the ivermectin and placebo groups was that thought most likely to be related to the Loctite super glue placed at the opening of the colostomy bag. Loctite has an ethyl cyanoacrylate base, and the vapors from the glue drying likely affected the ticks. However, it is possible that the ostomy glue placed around the ostomy bag may have contributed to mortality as well. We believe that the high mortality seen in the placebo group demonstrates a structural flaw in our study design. The ostomy bags were not securely attached to study subjects, as reflected in the large percentage of ticks that had escaped by day 3. The lack of a tight seal between the ostomy bag and the skin allowed water and soap to enter the bag when study subjects showered. Most of the ticks accumulated at the bottom of the ostomy bag. In our experiment, the opening of the ostomy bag was caudal and by orientating the opening cephalad, we would have kept the ticks closer to the skin rather than the ostomy pouch. Study subjects applied athletic tape around the perimeter of the ostomy bag but that was not completely effective at creating a tight seal against the skin.

Conclusions

Our data demonstrate that no definitive conclusion can be made regarding *I. scapularis* morbidity and mortality after exposure to ivermectin. Further research is needed to determine the optimal mechanism to safely attach ticks to humans.

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