Experimental evidence for usefulness of real time high resolution ultrasonography in evaluation of efficacy of macrofilaricidal agent

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In the present study, a CDRI candidate antifilarial agent compd 82/437 was evaluated for macrofilaricidal activity by ultrasonography (USG), a direct, safe and non-invasive sequential monitoring system using experimental filarial infection. Acanthocheilonema viteae adult filarial parasites were detected using USG in rodent host (Massomys natalensis). The motility of adult filariids was monitored by observing peculiar echo-patterns or filaria dance sign. CDRI candidate drug 82/437 was administered in A. viteae-infected mastomys. Drug action was monitored in situ at weekly intervals till day 42 post-treatment with USG. After treatment, parasites appeared sluggish on day 28 and on day 35, no mobile echo-pattern was observed suggesting drug action. Findings were confirmed by recovering the dead parasites from treated animals.

Ultrasoundography (USG) in medicine has become a vital tool for diagnostic and therapeutic purposes in parasitic diseases. Recently, use of USG to reveal the presence and location of live adult filariae in lymphatic tissue has opened up a new horizon in the study of the vector-borne parasitic disease, filariasis. Till recently, ultrasound had been used in the context of parasitic diseases, to visualize liver damage from schistosomiasis, to locate deep nodules in onchocerciasis and to detect migratory Ascaris worms. Recently, WHO has recommended the use of this technique, to check the motility of worms and also recommended its use in assessing the efficacy of antifilarial agents before treatment and to see if they die after treatment. Nevertheless, there is an urgent need to know the minimum dose for effective cure by candidate drugs in drug development programmes. By USG, visualizing of parasites and evaluation of drug efficacy are more convenient and there is no harm towards animals involved. The present study was carried out to evaluate the potential of USG to localize and image the adult parasites of Acanthocheilonema viteae dwelling in the subcutaneous tissues in the experimental host M. natalensis. USG was also used to detect the chemotherapeutic efficacy of the antifilarial agent, CDRI compd 82/437 (a macrofilaricidal agent) in experimental filariasis.

The studies carried out were the induction of experimental filarial infection in the rodent host M. natalensis. Mastomys were infected by injecting subcutaneously 50 infective larvae (L1) of A. viteae obtained from the infected vector Ornithodoros moubata. Animals at 75 days (post L3 inoculation) showing progressive rise in microfilariae were selected for the present study.

CDRI compd 82/437, a potent macrofilaricidal agent was used in the present study. The ED100 was used as the effective dose in A. viteae/mastomys model. Group I consisted of 6 uninfected healthy animals. Group II (12 infected animals) received only the vehicle, which served as control. Group III containing 24 infected animals in two batches was treated with CDRI compd 82/437 at a dose of 50 mg/kg i.p. for 5 days. The drug was prepared in distilled water in the presence of 1% Tween 80.

Animals of batch 1 (group III) were sequentially examined at weekly intervals, i.e. from day 8 to day 42 of treatment while animals of batch 2 (group III) were sacrificed at weekly intervals (two animals each on days 8, 14, 21, 28, 35 and 42 of treatment) to assess the condition of worms. The whole set of experiments was repeated thrice.

The microfilaraemia of treated and control animals was assessed by taking five cubic millimeter of tail blood from each animal before starting the treatment and thereafter at weekly intervals up to the completion of the observation period, i.e. day 42. The intensity of microfilaricidal action in any individual animal was expressed as a per cent change in population of microfilariae over pretreatment levels. To assess macrofilaricidal action, animals were sacrificed under deep anaesthesia and motility of the parasites was examined in normal saline.

Real time ultrasonography was performed using a 10 MHz transducer (Ultramark 4, ATL, USA). Imaging was recorded on a video recorder and videographic printer (UP870 MD).

USG was initially performed on adult A. viteae parasites, by keeping them inside a dialysis bag containing nutrient medium HBSS with the aim to monitor the nature of movements and echogenicity of the adult parasites. USG studies were later performed in normal, infected and infected-treated hosts. Animals were anaesthetized by injecting thiopentone intraperitoneally (Intraval sodium, May and Baker Pharmaceuticals, UK) at a dose of 50 mg/kg prior to USG examination. The region of interest, i.e. cervical region (due to availability of maximum parasites) was selected for monitoring the nests of adult parasites.

Ex vivo examination of adult A. viteae parasites showed filaria dance sign (FDS)/mobile echo-patterns due to motility of the parasites (Figure 1). In vivo examination of infected hosts demonstrated the hyperechoic dermis,
hypoechoic hypodermis and hypo and hyperechoic subcutaneous tissue layers (Figure 2). The dermal layer typically measured 2–3 mm thick. The thickness of the hypodermis varied considerably depending on the region of the body. The subcutaneous fat in the hypodermis appeared less echogenic as the ratio of fat to lean body mass increased. Fibrous septa within thin layers was hyperreflective relative to the fat lobules. As they became more widely separated by larger fat lobules, echogenicity of the fat decreased\(^\text{23}\) (Figure 2). The connective tissue network anchored the skin to the underlying fascia while allowing a moderate-free motion between the layers. A regular cardiac pulse was observed which was very intense when the transducer was kept on the region close to the heart.

The USG examination of \textit{A. viteae}-infected hosts showed prominent peculiar mobile echo-patterns or FDS (arrows) below the dermal layer and also embedded in the subcutaneous tissues (Figure 3). The parasites measured (width) less than 1 mm (as accurate measurement below 1 mm was beyond the capability of the system). The actual length of these parasites was 2–3 cm (males) and 5–6 cm (females) and width 0.1–0.3 mm. Parasites appeared as linear hypoechoic structures with hyperechoic edges. No such peculiar echogenic, mobile points were observed in the normal host during real time studies.

**Figure 1.** Ultrasound images of the adult parasites inside dialysis bag showing mobile linear central hypoechoic structure with hyperechoic edges (arrows).

**Figure 2.** Ultrasound images from the cervical region of the normal mastomys showing hyperechoic dermis (D), hyperechoic hypodermis (H) and hypo and hyperechoic subcutaneous tissues (S).

**Figure 3.** Ultrasound images from the cervical region of \textit{A. viteae} infected mastomys showing dermis, hypodermis and subcutaneous tissues, adult parasites appear as mobile echogenic points below the dermal layer and also embedded in the subcutaneous tissues (arrow heads).

**Figure 4.** Ultrasound images from the cervical region of treated, infected mastomys post 28 days of drug therapy showing uncoiling of the parasites (arrow heads).
Table 1. Effect of compd 82/437 on microfilariae in mastomys infected with *A. viteae*

<table>
<thead>
<tr>
<th>Group of animals</th>
<th>Microfilariae count pretreatment (Mean ± SD)</th>
<th>Change (Mean ± SD) in microfilarial level on days post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected untreated (control)</td>
<td>150 ± 5.0</td>
<td>165 ± 2.5</td>
</tr>
<tr>
<td>Treated</td>
<td>140 ± 6.9</td>
<td>208 ± 12.0</td>
</tr>
<tr>
<td>Significance</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Effect of compd 82/437 on worm recovery in mastomys infected with *A. viteae*

<table>
<thead>
<tr>
<th>Day of sacrifice</th>
<th>No. of live worm recovery (Mean ± SD)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>19 ± 5.1</td>
<td>18 ± 5.7</td>
</tr>
<tr>
<td>14</td>
<td>17 ± 4.9</td>
<td>16 ± 4.3</td>
</tr>
<tr>
<td>21</td>
<td>17 ± 5.5</td>
<td>15 ± 5.0</td>
</tr>
<tr>
<td>28</td>
<td>20 ± 1.57</td>
<td>14 ± 6.6</td>
</tr>
<tr>
<td>35</td>
<td>18 ± 5.5</td>
<td>0</td>
</tr>
<tr>
<td>42</td>
<td>16 ± 4.1</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3. Comparative observations of *A. viteae* parasite motility by surgical and USG examination

<table>
<thead>
<tr>
<th>Observation period (in days)</th>
<th>Motility</th>
<th>USG examination</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>++</td>
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<td>8</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>14</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>21</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>28</td>
<td>+</td>
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<tr>
<td>35</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>42</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+++ Hyperactive; ++ Active; + Sluggish and uncoiled; -, No movement.

(Figure 2). All 36 infected animals showed FDS due to the motility of the parasites.

Infected animals (group III consisting of 2 batches) which on USG examination showed FDS, were treated with CDRI compd 82/437 (a macrofilaricidal agent) at a dose of 50 mg/kg i.p. for 5 days. The antifilarial profile of CDRI compd 82/437 is shown in Tables 1 and 2.

The USG examinations were carried out at weekly intervals (i.e. on days 8, 14, 21, 28, 35 and 42) post treatment in both the batches of group III. On days 8 and 14, there was minor difference in the intensity of the FDS or echogenic patterns as observed in the cervical region of the infected-treated hosts. On day 8, the FDS showed highly motile adult parasites in comparison to day 14 as well as that of untreated-infected animals when examined by USG. On day 21, the FDS had slowed down and the body of the adult *A. viteae* parasites appeared uncoiled showing signs of drug effect. On day 28, the movement of the parasites or the echogenic pattern was very sluggish and the body of the parasites remained uncoiled (Figure 4). On day 35, there was no movement or FDS of the parasites, thereby showing the macrofilaricidal action of compd 82/437. The region of interest, i.e. cervical region of infected, treated hosts showed consistent ultrasound images from one examination to the next. The results were confirmed by sacrificing the animals of batch 2 (group III) at weekly intervals, i.e. on days 8, 14, 21, 28, 35 and 42 post treatment. No necropsy was carried out in animals of batch 1 (group III). Comparative observation of the condition of adult parasites was carried out by USG and surgical examinations (Table 3).

In case of human lymphatic filariasis, the major lacuna lies in non-invasive evaluation of chemotherapeutic efficacy of macrofilaricidal drugs *in situ*. Recently, a remarkable advance has been made in localization of live adult worms of *W. bancrofti* in human hosts6. Nests of highly motile adult worms in the scrotal lymphatics of infected microfilaraeemic subjects have been clearly seen by USG6.

Lymphatic abnormalities caused by filarial infection have also been investigated by two other noninvasive imaging techniques, lymphoscintigraphy and magnetic resonance imaging (MRI) in experimental and human filariasis with some success24-28. However, MRI is difficult to interpret alone and often requires, in addition to MRI, light scanning and video microscopy to identify live adult worms27.

The recent demonstration of dancing parasites of *W. bancrofti* by USG has opened up a new possibility in the study of filarial infection. It is worth mentioning that standardization and application of USG in assessing the motility of parasites before and after antifilarial therapy has been recommended by WHO16. Due to the noninvasive nature of ultrasound, it has the potential to be a tool in which to follow parasitic activity following drug treatment.

The main intention of the present study was to see non-invasively if the parasites die after treatment with
antifilarial agent, macrofilaricidal, in experimental filariasis. The subcutaneously dwelling adult parasites of *A. vitae* in rodent host *M. natalensis* were localized and imaging was done by ultrasonography. The effect of the drug, CDRI compd 82/437 was monitored by visualizing the slow death of adult parasites *in situ*.

The prominent peculiar mobile echo-patterns or FDS of parasites inside the host proved to be useful in the observation of macrofilaricidal action of drug on the adult worms. Earlier studies carried out with this drug have shown that it acts by inhibiting the glucose uptake by the filarial parasites and also inhibits the activity of antioxidant enzymes like superoxide dismutase (SOD), catalase and glutathione-s-transferase (GST) of filarial parasites through which the parasites defend themselves from the attack of the host’s defence system[29–32].

The adult *A. vitae* parasites as examined by USG were visualized below the dermis and were also found embedded in the subcutaneous tissue consisting of connective tissue and fat cells. The parasites were highly motile and the mobile echo-patterns were observed at regular intervals after drug therapy. The intensity of these mobile echo-patterns or FDS diminished during weekly observations. On day 28, the parasites' body seemed uncoiled and the movement was very sluggish while on day 35, there was no movement or FDS at all, thereby indicating death of adult parasites. The sonological changes observed in our study, thus, correlates well with the parasitological and surgical examinations. The USG images are also useful in showing the severity of infection and efficacy of the macrofilaricidal drug in experimental filariasis.


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