Retinoid Expression in Onchocercal Skin Disease: Pilot Study

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ABSTRACT: Based on the observation that the parasite *Onchocerca volvulus* selectively absorbs vitamin A from the host, and the known toxicity of vitamin A in higher concentration, it was hypothesized that dying microfilariae (mf) release their stores of vitamin A (retinoids) into the host circulation in toxic concentrations, inducing the signs and symptoms of onchocerciasis. We conducted a pilot study to test the hypothesis in Songea communities in Southern Tanzania, where mass drug administration with ivermectin had not been implemented by the time of the survey. The specific aim was to evaluate the correlation between the diagnosis of onchocerciasis and increased levels of retinoic acid at infection sites. The analysis was performed by determining copy numbers of a genome of *O volvulus* present in skin snip samples of persons with onchocerciacis, and correlating these numbers with expression levels of retinoic acid receptor-α (RAR-α), which is inducible by retinoic acid. Total DNA and RNA were extracted from each of 25 mf-positive and 25 mf-negative skin samples and evaluated using quantitative polymerase chain reaction with appropriate negative controls. Analysis of the samples, adjusted with glyceraldehyde 3-phosphate dehydrogenase gene levels, revealed that most samples with detectable RAR-α transcripts had higher levels of RAR-α expression than the assay control. However, the quality and number of samples were insufficient for statistical analysis. Fold data on the expression levels of both *O volvulus* DNA and RAR RNA suggested a possible trend toward higher relative RAR-α expression in samples with higher levels of *O volvulus* DNA ($r^2 = 0.25$, $P = .079$). Evidence of a contribution of vitamin A to the pathology of onchocerciasis thus remains elusive. Future studies on the role of retinoids in onchocerciasis will require larger groups of participants as well as careful monitoring of the cold chain and tissue storage procedures in view of the sensitivity of vitamin A to heat and light.

KEYWORDS: Onchocerciasis, skin, eye, retinoids, hypervitaminosis A, pathophysiology

Introduction

Onchocerciasis ("river blindness") is a chronic and insidious but nonfatal ocular and dermatological filarial disease transmitted by black flies (*Simulium* sp.). An estimated 37 million people are infected globally with the parasite *Onchocerca volvulus* and about 270 000 are blind.[1] The adult filarial nematode can survive for more than a decade in the subcutaneous nodules of affected individuals, releasing millions of microfilariae (mf) that trigger debilitating itching and visual impairment.[2–4] The disease causes lifelong suffering as well as catastrophic socioeconomic problems in affected communities.

In Tanzania, where endemic regions include the series of block mountains that run in an arc-like chain from Northeast Tanzania to Northern Malawi, and from the Tanga Region to Ruvuma and southwestern Mbeya, the total population in meso/hyperendemic areas in 2007 was estimated to be about 2 million.[5] The epidemiology of the disease has changed in recent years due to local environmental and global changes in climate and human populations as well as parasite and vector dynamics and heterogeneity in the human host response.[6–8] In endemic areas, where 40% to 50% of adults are symptomatic, the disease has caused entire villages to be abandoned. The effectiveness of the main treatment—ivermectin—is also declining,[9] with alarming indications of resistance to the drug.[10] An urgent need exists, therefore, to understand the pathogenesis of the disease and to develop new treatment strategies.[11]

The major symptoms of the disease, both dermatological and ocular, are associated with localized inflammatory reactions to dead or dying mf and the products of endosymbiontic bacteria (*Wolbachia*) released into the circulation.[7,8] However, the precise mechanisms of disease are uncertain. A potential clue to understanding the pathogenesis is that adult *O volvulus* worms selectively absorb vitamin A from the host, causing parasite retinoid concentrations to be considerably higher than those of surrounding host tissues. This was shown in a study of 4 batches of adult worms derived from the onchocercomata of 4 different

individuals,[12] in which the median tissue retinol concentration was 3.8 μg/g (range: 0.5-11.9 μg/g) (12.6 IU/g [range: 1.6-39.7 IU/g]), ie, 8 times higher on average than in host skin, but lower than normally found in fresh liver (~240 μg/g [800 IU/g]). Blood samples and skin biopsies from 8 patients with onchocerciasis and onchocercomata, and 4 clinically uninfected controls showed that the mean plasma retinol concentration in the patients was 40 μg/100 mL (range: 20-60 μg/100 mL) (1.4 IU/mL [range: 0.8-1.9]) which in every case was above the critical (deficiency) level of 10 μg/100 mL (0.3 IU/mL). In the skin, however, the median retinol concentration of the patients was 0.45 μg/g (range: 0.09-2.1) (1.5 IU/g [range: 0.3-7.0]) compared with 0.3 μg/g (range: 0.18-0.84) (1.2 IU/g [range: 0.6-2.8]) in the 4 controls, suggesting increased concentrations of retinol in the skin of cases versus controls. However, this conclusion remains speculative, as the numbers were small and the difference was not statistically significant. Based on these early findings and the assumption that the mf, like the adult worms, similarly contain high concentrations of retinoid, Mawson and WaKabongo[13] proposed that on the death of thousands of mf on a daily basis, retinoid compounds are released back into the circulation and rise to toxic concentrations in the affected tissues, producing localized and systemic manifestations of hypervitaminosis A recognized as the signs and symptoms of the disease.

Retinoids (the collective term for vitamin A and its congeners) are fat-soluble signaling molecules that are essential in low concentration for numerous body functions but can be cytotoxic, prooxidant, mutagenic, and teratogenic in higher concentration.[14] Retinoids derive mostly from dietary sources, and about 80% of tissue vitamin A is stored in the liver. Retinol is transported from the liver to the target tissues as retinol-binding protein (RBP), where it is converted to retinaldehyde and subsequently to retinoic acid (RA). The latter serves as a ligand for the retinoic acid receptors (RARs) and the retinoid X receptors, each of which has 3 isoforms—α, β, and γ. These receptors form heterodimers and induce the activation of numerous genes.[15,16]

This article reports the results of a pilot study undertaken to test the retinoid toxicity hypothesis in Songea communities in Southern Tanzania, where mass drug administration with ivermectin had not been implemented by the time of the survey. The specific aim was to determine whether there was an association between *O volvulus* infection and RA in affected skin by determining copy numbers of a genome of *O volvulus* present in skin samples of potential onchocerciacis cases, and by correlating these numbers with expression levels of RAR (RAR-α), which is inducible by RA.

Materials and Methods

Study area

Songea, Namtumbo, and Mbinga were selected as study sites based on their levels of disease transmission endemicity (ie,

meso, hyperendemicity: Songea; hypoendemicity: Mbinga and Namtumbo). The largest onchocerciacis focus area in southwestern Tanzania is Ruvuma, which comprises the 3 study sites of Songea (which lies between latitudes 1.64S and longitudes 35.64E), Mbinga (situated between latitudes 10.94S and longitudes 35.00E), and Namtumbo (which lies between latitudes 10.47S and longitudes 36.13E). The Ruvuma focus borders Mozambique to the south and Lake Nyasa to the West, covers around 15 000 km², and is characterized by a number of river systems that flow from and traverse the Livingstone mountain range into Lake Nyasa. These river systems provide ideal *Simulium* black fly vector breeding sites and maintain onchocerciasis transmission within this large area.

Study design and sampling procedures

The study began as a community survey designed to identify individuals with onchocercal skin disease (OSD) and controls. A total of 106 adult men and women aged 18 to 65 years (53 cases and 53 controls) were initially identified and invited to participate in the study. Potential cases presenting with OSDs, including acute and chronic papular onchodermatitis (CPOD), atrophy, depigmentation (DSM), and/or nodules, diagnosed by a physician experienced in the clinical diagnosis of OSD, were confirmed by microscopy (skin snips or shavings positive for mf). Individuals who were mf-negative and lacking signs and symptoms of clinical disease served as controls (n = 53) and were mainly recruited from areas considered hypoendemic or with no active transmission. Had chronic debilitating diseases, were immune-compromised in any way or, within the past month, had consumed alcohol, or used dietary supplements containing vitamin A (Table 1).

The study was approved by the Institutional Review Boards of Jackson State University, Jackson, MS, USA, and the Medical Research Coordinating Committee (MRCC) of the National Institute for Medical Research in Tanzania (Ref NIMR/HQ/R.8c/Vol II/59 2013). Oral and written informed consent was obtained from all study participants.

Specimen collection and processing

Skin snips were taken from right and left iliac crests, pelvic girdles, and buttocks, collected in 96-well microtiter plates of 200 μL with 0.9% normal saline, and sealed with parafilm. All specimens were labeled in code and shipped frozen to ACGT, Inc. (Wheeling, IL, USA) for processing.

Assay design

The *O volvulus* genome copy number assay was designed by inputting known *O volvulus* genomic sequences into an Applied Biosystems (Foster City, CA, USA) online minor groove binder assay design tool. Several possible primer/probe combinations were evaluated for recognition of non-*O volvulus* sequences,

Table 1. Demographic characteristics of the study participants recruited in Ruvuma.

VARIABLE	CASES (N=53)	CONTROL (N=53)	*P* VALUE
Sex			
Male	37 (69.8)	26 (49.1)	.03
Female	16 (30.2)	27 (50.9)	
Age, y, mean (SD)	50.8 (12.5)	45.0 (14.3)	.02
Education			
None	20 (37.7)	14 (26.4)	.02
Primary	32 (60.4)	31 (58.5)	
Secondary	1 (1.9)	8 (15.1)	
Body mass index			
Mean (SD)	21 (2.8)	24.8 (4.4)	<.001
Occupation, No. (%)			
Peasants	51 (96.0)	45 (84.9)	
Clinical SS, No. (%)			
APOD	19 (35.9)	0 (0)	
CPOD	26 (49.1)	0 (0)	
DPM	16 (30.2)	0 (0)	
ATP	18 (34.0)	0 (0)	
Itching	49 (92.4)	0 (0)	

Abbreviations: APOD, acute papular onchodermatitis; ATP, atrophy; CPOD, chronic papular onchodermatitis; DPM, depigmentation; SD, standard deviation; SS, symptoms and signs.

either human or nematodes other than *O volvulus*, by running primer sequences against all available genomes on the National Center for Biotechnology Information site. The chosen Ovol2 assay had the least homology to non-*O volvulus* genomic sequences.

DNA/RNA extraction and complementary DNA synthesis

Chosen skin snip samples were added to a mixture of lysis buffer and glass beads and vortexed vigorously for 5 minutes. DNA and RNA were then individually extracted using the Fisher Scientific, (Cat. #444038) 'NanoDrop spectroscopy', NanoDrop ND-8000 UV/Vis spectrophotometer) and '2100 Bioanalyzer' (Agilent Technologies, Waldbronn, Germany, Part Number: G2946-90004). Complementary DNA (cDNA) was synthesized using a SuperScript III Kit, according to the manufacturer's instructions.

Quantitative polymerase chain reaction and data analysis

A target-specific TaqMan assay for *O volvulus* genomic sequence, for RAR-α transcripts, and for the genomic sequence of human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene, was obtained from Applied Biosystems Inc. (ABI). Optimized assay conditions were then established for the copy number assay using several of the provided samples and human genomic DNA from blood as a negative control. Both PCR targets were amplified from each test sample genomic DNA (gDNA) in triplicate using the 7900HT Sequence Detection System (Applied Biosystems). A cycle threshold (Ct) value was generated for all target amplifications for each sample. The relative copy numbers of *O volvulus* genome (average copies/cell) in each sample was determined by comparing the Ct values of the *O volvulus* target sequence in skin samples with that of the negative control, after normalization with the GAPDH control. Both GAPDH and RAR-α targets were amplified from each test sample cDNA in triplicate using the 7900HT Sequence Detection System and the ABI assays. A Ct value was generated for all target amplifications for each sample. The relative expression levels of RAR-α transcripts in each sample were determined by comparing the RAR-α Ct values in skin samples with that of the negative control, after normalization with GAPDH expression level control. For those samples where both RAR-α expression and *O volvulus* genome levels were obtained, a correlation between the 2 metrics was evaluated with a scatterplot (see below).
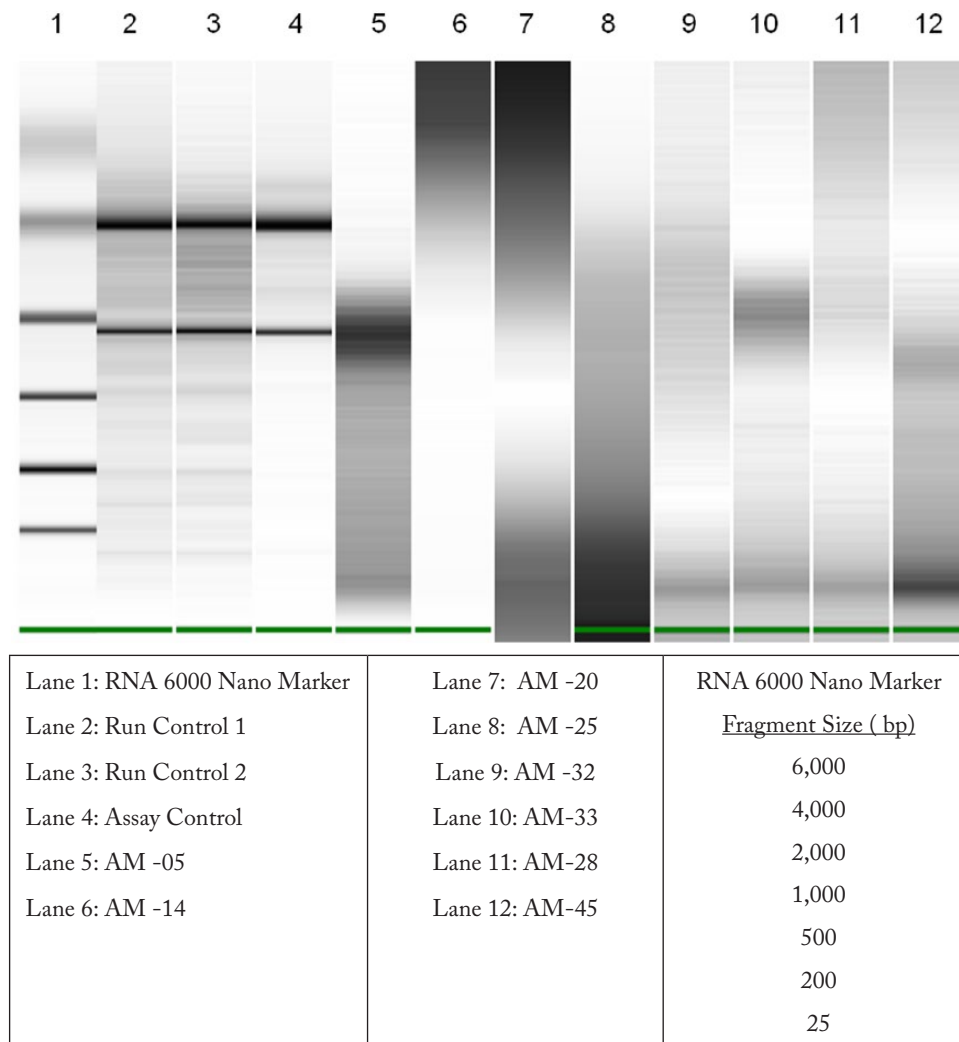
Lane 1: RNA 6000 Nano Marker	Lane 7: AM -20	RNA 6000 Nano Marker
Lane 2: Run Control 1	Lane 8: AM -25	Fragment Size (bp)
Lane 3: Run Control 2	Lane 9: AM -32	6,000
Lane 4: Assay Control	Lane 10: AM-33	4,000
Lane 5: AM -05	Lane 11: AM-28	2,000
Lane 6: AM -14	Lane 12: AM-45	1,000
		500
		200
		25

Figure 1. RNA quality results from the 2100 Bioanalyzer.

Results

A custom TaqMan assay for detection of *O volvulus* genomic DNA (Ovol2) was designed and synthesized at Applied Biosystems Inc. Additional TaqMan assays for detection of RAR-α expression and for normalizing human DNA and RNA levels (housekeeping gene GAPDH) were obtained as Assays-on-Demand from Applied Biosystems. Both genomic DNA and total RNA were extracted from the skin snip samples with the Fisher SurePrep RNA/DNA extraction kit. One in-house blood sample (not associated with this study) was also processed with this kit to obtain negative control human gDNA and RNA. Quality and quantity of genomic DNA recovered from the samples was evaluated with NanoDrop spectroscopy. Although the results varied significantly, all samples were submitted for quantitative polymerase chain reaction (qPCR) analysis. Similarly, RNA samples were analyzed with NanoDrop spectroscopy (Figure 1), and most appeared to have sufficient quantity of RNA for testing. A set of 8 RNA samples was evaluated with the 2100 Bioanalyzer for RNA quality/integrity (Figure 1). Although the assay control (extracted from whole blood) had good-quality RNA with an RNA integrity index (RIN) value of 9.6, the RNA samples from the skin snips had highly degraded RNA, with low RIN. Nevertheless, all RNA samples were used to prepare cDNA with the SuperScript III kit, (Invitrogen, Cat # 11752-050) using 7.5 ng/reaction of RNA (based on NanoDrop readings).

Genomic DNA samples were evaluated with the Ovol2 assay for the presence of *O volvulus* genomes. Relative parasite copy numbers were normalized to copy number of human genomic DNA as determined by the GAPDH assay and reported as fold averages ± SEM relative to the levels observed in the negative assay control (Figure 2). Average Ct values obtained for GAPDH assay showed that the total amount of human DNA detected was similar in most samples, with some exceptions (typically related to low DNA quantity and/or quality). The amounts of *O volvulus* DNA detected in the samples ranged from being undetectable in 11 of the samples to more than 1000-fold the background seen in the assay control (Figure 2). However, there was no clear correlation between the mf-positive or mf-negative groups and the detected levels of *O volvulus* DNA.

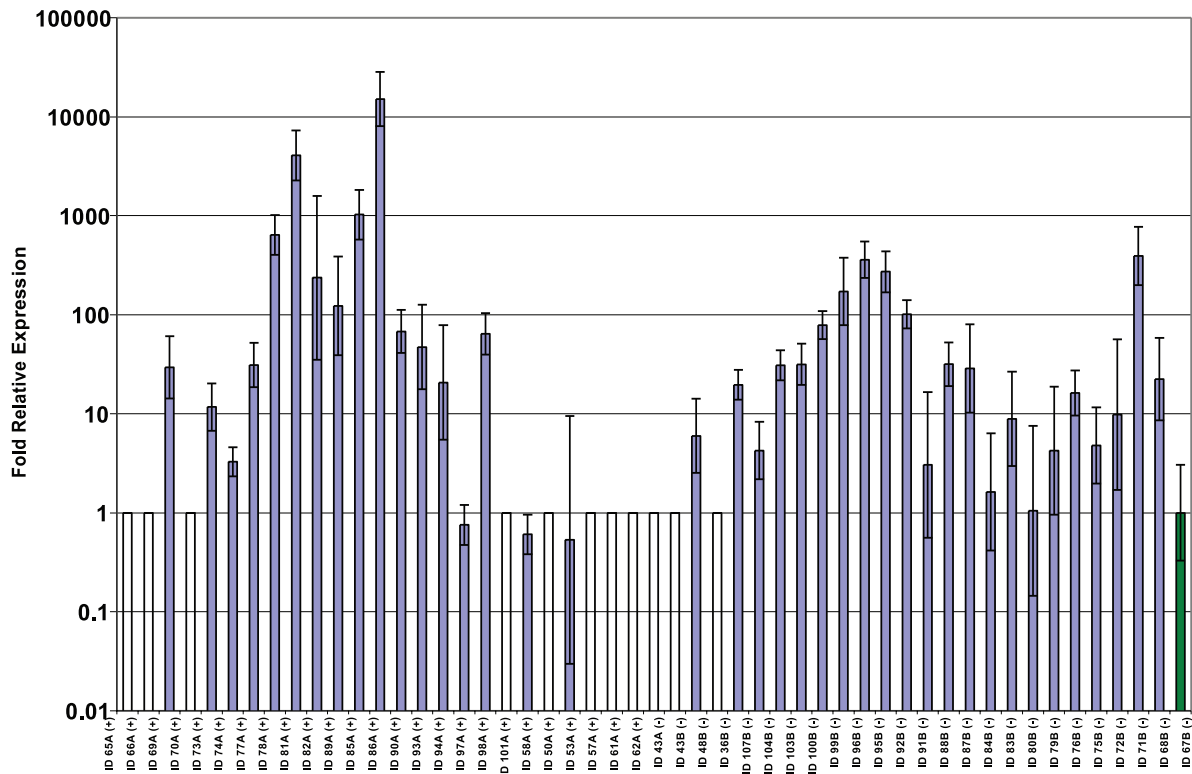All cDNAs prepared from the total RNA samples were evaluated for the expression of RAR-α transcripts, with the

Figure 2. Fold *Onchocerca volvulus* genomes relative to genomic DNA control (normalized with glyceraldehyde 3-phosphate dehydrogenase levels). (+) or (–) tags after sample IDs designate their association with positive and negative sample groups, respectively. Uncolored bars indicate that no average Ct was obtained for the Ovol2 assay for that sample. Ovol2 levels for the control sample have been adjusted to 1 and are highlighted in green.

relative expression levels normalized to the expression of GAPDH and reported as fold averages ± SEM relative to the levels observed in the negative assay control sample (Figure 3). Examination of the GAPDH Ct values showed that overall quality of the RNA was poor, with detectable levels of RAR-α expression observed in only 4 of the 25 mf-positive samples and 9 of the negative group samples. Even in those samples, the levels of GAPDH RNA were significantly lower than in the assay control. However, when RAR-α expression levels were adjusted with GAPDH levels, most of the samples with detectable RAR-α transcripts appeared to have higher levels of RAR-α expression than the assay control.

Clinical and demographic characteristics

As mentioned, skin snip specimens were collected from 53 mf-positive and 53 mf-negative individuals from Ruvuma, Tanzania and assayed at ACGT Inc. After being checked for quantity and quality of tissue, 25 mf-positive and 25 mf-negative specimens were found to have sufficient material to yield the necessary amount of nucleic acid to analyze for DNA/RNA. The remaining specimens could not be used due to deterioration or insufficient quantity of analyzable material. The laboratory then selected the most usable 25 mf-positive and 25 mf-negative samples for qPCR analysis.

Results of the *O volvulus* DNA microassay showed that 22 of the 25 mf-negative controls had elevated genomic fold levels, indicating unsuspected subclinical infection with *O volvulus*,

despite the absence of either visible mf under the microscope or signs of OSD, such as acute papular onchodermatitis, CPOD, DPM, atrophy, lichenified onchodermatitis, or nodules. Therefore, cases and controls were pooled and analyzed as one sample to evaluate a possible correlation between *O volvulus* DNA levels and RAR-α expression. Results for those samples are seen in Figures 4 and 5. Although there was no significant correlation between the levels of either *O volvulus* DNA and the mf-positive or negative groups or between the levels of RAR-α expression and the positive or negative groups, a possible trend was seen in the skin snip data suggesting an increase in RAR-α expression levels with increasing levels of *O volvulus* DNA ($r^2 = 0.25$, $P = .079$) (Figure 4). This relationship can be visualized more clearly in an *O volvulus* DNA/RAR-α expression scatterplot. However, it is somewhat tenuous, as evident by the low r^2 value of higher relative RAR RNA expression in samples with higher levels of *O volvulus* DNA ($r^2 = 0.25$, $P = .079$) (Figure 5).

Discussion

This pilot study represents a first attempt to test the retinoid toxicity hypothesis in patients with OSD. On this hypothesis, the major symptoms of severe itch and impaired vision are due to prolonged intermittent exposure of the skin and eyes to retinoids released into these tissues following the death of large numbers of mf on a daily basis.[13] Several lines of evidence support the retinoid toxicity hypothesis of onchocerciasis. First, *O volvulus* produce antigens with high-affinity binding protein for fatty acid and retinol (FAR); the Ov20 protein binds retinol and is
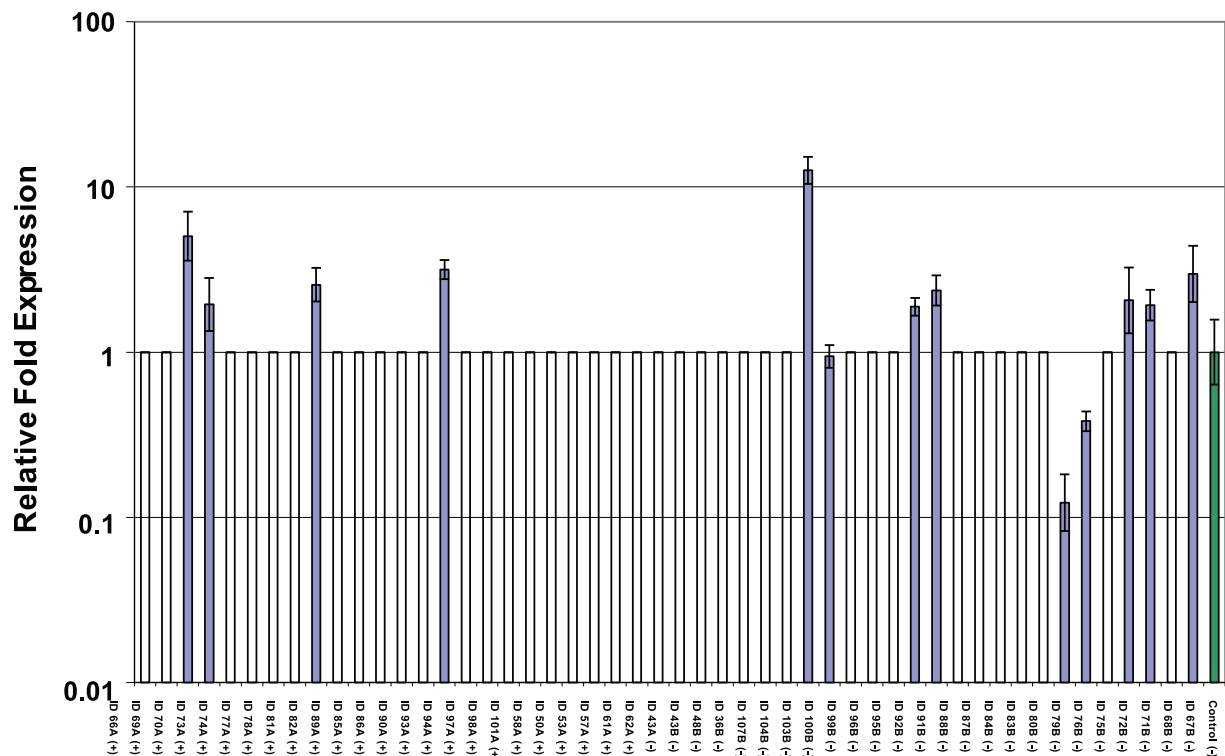
Figure 3. Fold expression of RAR-α relative to control sample (normalized with expression levels of GAPDH). GAPDH indicates glyceraldehyde 3-phosphate dehydrogenase; RAR-α, retinoic acid receptor-α.

(+) or (−) tags after sample IDs designate their association with positive and negative sample groups, respectively. Uncolored bars indicate that no average Ct was obtained for the retinoic acid receptor (RAR) and/or GAPDH assay for that sample. Retinoic acid receptor expression levels for the control sample have been adjusted to 1 and are highlighted in green.
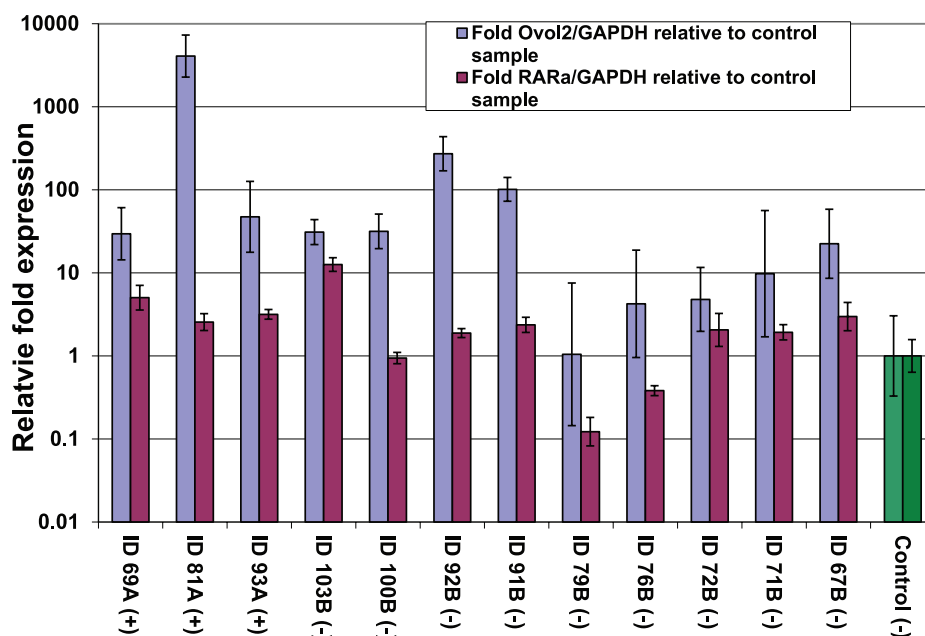

Figure 4. Correlation between relative levels of *Onchocerca volvulus* genome and RAR-α relative expression levels. GAPDH indicates glyceraldehyde 3-phosphate dehydrogenase; RAR-α, retinoic acid receptor-α.

(+) or (−) tags after sample IDs designate their association with positive and negative sample groups, respectively. Ovol2 and RAR relative levels for the control sample have been adjusted to 1 and are highlighted in green.

abundant in the body wall and in the developing larvae. Ov20 (now known as Ov-FAR-1) accumulates to high levels in the onchocercal nodule and is secreted in vivo. The comparative amounts of RA-binding protein found in *O volvulus* are also much higher than those of mammalian and avian origins.[17] Functional FAR are also secreted by adult hookworms, similar to Ov-FAR-1 from *O volvulus*, suggesting that FAR proteins secreted by parasitic nematodes are crucial to parasitism, possibly
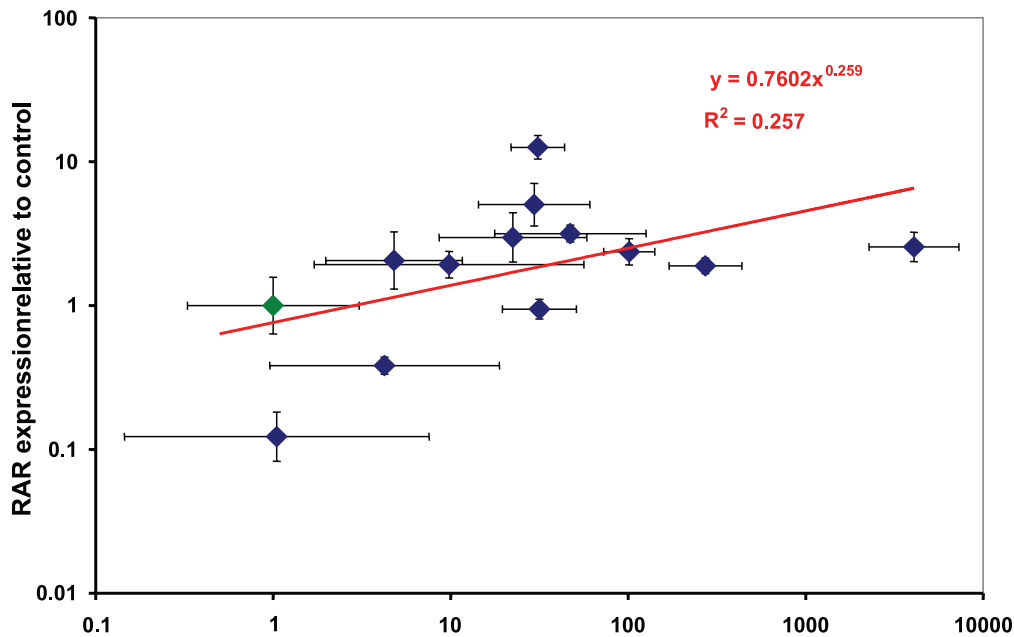
Figure 5. A scatterplot evaluation of the relationship between relative levels of *Onchocerca volvulus* DNA and RAR-α expression. RAR-α indicates retinoic acid receptor-α. *Onchocerca volvulus* genome copies relative to control. Control sample data point is highlighted in green.

having roles in acquiring signaling lipids from the hosts.[18] It has also been suggested that the release of parasite RBPs contributes to the skin and eye pathology by competing for the ligand and interfering with the host's transport mechanisms of retinol, ie, creating a localized state of vitamin A deficiency.[19]

Second, the common clinical features of onchocerciasis (eg, pruritus, musculoskeletal pain, bone changes, lethargy, and growth arrest in children) are similarly reported following excessive intakes of vitamin A or after prolonged treatment with retinoids.[20,21] Hallmarks of onchocerciasis are severe visual impairment and skin DSM. Ocular reactions to prolonged use of systemic retinoid therapy likewise include corneal opacities, disorders of refraction, and alterations in retina and optic nerve.[22,23] The manifestations of DSM in onchocerciasis include areas of complete pigment loss (ie, vitiligo) with islands of normal skin pigment ("leopard skin"). Consistent with the hypothesis, mice homozygous for the vitiligo (Mitf-vit) mutation experience progressive degeneration of the retina as well as uneven pigmentation of the retinal pigment epithelium (RPE), associated with significantly increased retinyl ester concentrations in eyes and liver. Retinyl palmitate is increased 5-fold in the eyes of affected mice at 10 weeks postnatally and increased 3-fold at 22 weeks of age, whereas plasma retinol levels remain in the normal range.[24] In the mutant RPE, retinyl ester levels are significantly elevated 4-fold by postnatal week 8.[25] By 10 weeks of age, retinaldehyde dehydrogenase and RA are significantly elevated in the neural retina of Mitf-vit mice relative to controls. The suggestion that increased RA could contribute to the retinal degeneration of Mitf-vit mice by inducing apoptosis[26] is supported by other observations indicating that retinoids can induce apoptosis.[27,28] All-*trans*-RA also causes moderate to complete DSM of Yucatan swine skin.[29] The appearance of skin DSM and severe visual impairment in these animals, in association with increased retinyl ester and RA concentrations in skin and eyes, suggests that similar processes may be occurring in onchocerciasis.

Third, the hypothesis could account in part for the therapeutic efficacy of the widely used antifilarial drug ivermectin. Although ivermectin is known to interact with postsynaptic glutamate-gated chloride channels (GluCl), resulting in paralysis of the mf,[30] the therapeutic efficacy of ivermectin remains only partially understood.[9] Consistent with the present hypothesis, ivermectin competes efficiently with retinol for retinol-binding sites on parasite RBP and has a higher affinity for parasite RBP than retinol; a correlation also exists between the binding affinities of ivermectin analogues and their antiparasitic action.[31,32] The therapeutic action of ivermectin could therefore be due to interference with parasite vitamin A metabolism. The drug does not kill the adult worms and there are typically no symptoms following treatment. This is consistent with the retinoid toxicity hypothesis because symptoms would be expected only if the adult nematodes were killed in large numbers and their contents released simultaneously. Unlike certain synthetic retinoids, which have a long half-life and accumulate over time,[33] all-*trans*-RA is rapidly metabolized and not stored in the liver or other organs.[34] It is rather the duration and "dose" of exposure of the host tissues to daily-released retinoids that may account for the postulated state of retinoid toxicity associated with onchocerciasis.

In this initial study, we sought to test the hypothesis by determining the association between *O volvulus* DNA and RAR RNA expression. Although successfully executed in the field, the study and its conclusions are limited due to deterioration of many samples prior to their arrival in the United States. Evidence of a contribution of vitamin A to the pathogenesis of onchocerciasis thus remains elusive. Future studies on the role of retinoids in onchocerciasis will require larger groups of patients and controls as well

as careful monitoring of the cold chain and tissue storage in view of the sensitivity of vitamin A to heat and light.[35]

Conclusions

A real-time PCR TaqMan assay was successfully developed for the detection of *O volvulus* genomic DNA levels in human samples. Although the assay was demonstrated to have low background in control human DNA sample, it lacked a validated positive control (*O volvulus* DNA target) to quantify its performance. As actual *O volvulus* genomic DNA remains unavailable, a recommendation would be to synthesize the assay target region in a plasmid, to be used as a normalized copy number control. Both DNA and RNA were extracted from the skin snip samples. However, the quality of RNA was significantly degraded, which negatively affected the validity and the interpretation of the results. The use of RNA preservation reagents such as RNA later is highly recommended in future field studies for collecting, preparing, and storing tissue samples for RNA analysis. No obvious correlation between levels of *O volvulus* DNA and sample assignment to either mf-positive or mf-negative groups was seen, but a possible trend suggesting increasing RAR expression levels with increasing levels of *O volvulus* DNA was noted. However, further studies are needed to confirm the validity of this correlation.

Author Contributions

ARM and WHM designed the study and drafted the paper, and WHM supervised the field work; ADP assisted with data analysis; WHM assisted with data analysis and drafting the paper; AKK, FF and AKK contributed to the field work; and SR analyzed the skin samples.

REFERENCES

1. Tada I. 3) Onchocerciasis. *Trop Med Health*. 2015;43:57–60.
2. Crump A, Morel CM, Omura S. The onchocerciasis chronicle: from the beginning to the end? *Trends Parasitol*. 2012;28:280–288.
3. Taylor MJ, Hoerauf A, Bockarie M. Lymphatic filariasis and onchocerciasis. *Lancet*. 2010;376:1175–1185.
4. African Programme for Onchocerciasis Control (APOC). Final communiqué of the 11th session of the Joint Action Forum (JAF) of APOC, Paris, France; December 6-9, 2005. http://www.who.int/apoc/about/structure/jaf/jaf10_final_communique.pdf.
5. African Programme for Onchocerciasis Control (APOC). United Republic of Tanzania Onchocerciasis status. http://www.who.int/apoc/countries/tza/en/. Published 2007.
6. Makunde WH, Salum FM, Massaga JJ, Alilio MS. Clinical and parasitological aspects of itching caused by onchocerciasis in Morogoro, Tanzania. *Ann Trop Med Parasitol*. 2000;94: 793–799.
7. Tamarozzi F, Halliday A, Gentil K, Hoerauf A, Pearlman E, Taylor MJ. Onchocerciasis: the role of *Wolbachia* bacterial endosymbionts in parasite biology, disease pathogenesis, and treatment. *Clin Microbiol Rev*. 2011;24:459–468.
8. Brattig NW, Rathjens U, Ernst M, Geisinger F, Renz A, Tischendorf FW. Lipopolysaccharide-like molecules derived from *Wolbachia* endobacteria of the filarial *Onchocerca volvulus* are candidate mediators in the sequence of inflammatory and antiinflamatory responses of human monocyte. *Microbes Infect*. 2004;2:1147–1157.
9. Laing R, Gillan V, Devaney E. Ivermectin—old drug, new tricks? *Trends Parasitol*. 2017;33:463–472.
10. Lustigman S, McCarter JP. Ivermectin resistance in *Onchocerca volvulus*: toward a genetic basis. *PLOS Negl Trop Dis*. 2007;1:e76.
11. Hotez PJ. Control of onchocerciasis. The next generation. *Lancet*. 2007;369:1979–1980.
12. Stürchler D, Wyss F, Hanck A. Retinol, onchocerciasis and *Onchocerca volvulus*. *Trans R Soc Trop Med Hyg*. 1981;75: 617.
13. Mawson AR, WaKabongo M. Onchocerciasis-associated morbidity: hypothesis. *Trans R Soc Trop Med Hyg*. 2002;96:1–2.
14. de Oliveira MR. The neurotoxic effects of vitamin A and retinoids. *Anais da Academia Brasileira de Ciências*. 2015;87:1361–1373 (Annals of the Brazilian Academy of Sciences). http://dx.doi.org/10.1590/0001-3765201520140677.
15. D'Ambrosio DN, Clugston RD, Blaner WS. Vitamin A metabolism: an update. *Nutrients*. 2011;3:63–103.
16. O'Byrne SM, Blaner WS. Retinol and retinyl esters: biochemistry and physiology. *J Lipid Res*. 2013;54:1731–1743.
17. Kennedy MW, Garside LH, Goodrick LE, et al. The Ov20 protein of the parasitic nematode *Onchocerca volvulus*: a structurally novel class of small helix-rich retinol-binding proteins. *J Biol Chem*. 1997;272:29442–29448.
18. Rey-Burusco MF, Ibáñez-Shimabukuro M, Gabrielsen M, et al. Diversity in the structures and ligand-binding sites of nematode fatty acid and retinol-binding proteins revealed by Na-FAR-1 from *Necator americanus*. *Biochem J*. 2015;471:403–414.
19. Bradley JE, Nirmalan N, Klager SL, Faulkner H, Kennedy MW. River blindness: a role for parasite retinoid-binding proteins in the generation of pathology? *Trends Parasitol*. 2001;17:471–475.
20. Silverman AK, Ellis CN, Voorhees JJ. Hypervitaminosis A syndrome: a paradigm of retinoid side effects. *J Am Acad Dermatol*. 1987;16:1027–1039.
21. de Oliveira MR. Vitamin A and retinoids as mitochondrial toxicants. *Oxid Med Cell Longev*. 2015;2015:140267.
22. Safran AB, Halioua B, Roth A, Saurat J-A. Ocular side effects of oral treatment with retinoids. In: Saurat J-A, ed., *Retinoids: 10 Years On*. Basel, Switzerland: Karger; 1991:315–326.
23. Turno-Kręcicka A, Grzybowski A, Misiuk-Hojło M, Patryn E, Czajor K, Nita M. Ocular changes induced by drugs commonly used in dermatology. *Clin Dermatol*. 2016;34:129–137.
24. Smith SB, Duncan T, Kutty G, Kutty RK, Wiggert B. Increase in retinyl palmitate concentration in eyes and livers and the concentration of interphotoreceptor retinoid-binding protein in eyes of vitiligo mutant mice. *Biochem J*. 1994;300:63–68.
25. Evans BL, Smith SB. Analysis of esterification of retinoids in the mouse retinal pigmented epithelium of the Mitf-vit (vitiligo) mutant mouse. *Mol Vis*. 1997;3:11.
26. Duncan T, Swint C, Smith SB, Wiggert BN. Levels of retinoic acid and retinaldehyde dehydrogenase expression in eyes of the Mitf-vit mouse model of retinal degeneration. *Mol Vis*. 1999;5:9.
27. Watabe H, Soma Y, Ito M, Kawa Y, Mizoguchi M. All-trans retinoic acid induces differentiation and apoptosis of murine melanocyte precursors with induction of the micropthalmia-associated transcription factor. *J Invest Dermatol*. 2002;118:35–42.
28. Hursting SD, Shen JC, Sun XY, Wang TT, Phang JM, Perkins SN. Modulation of cyclophilin gene expression by N-4-(hydroxyphenyl) retinamide: association with reactive oxygen species generation and apoptosis. *Mol Carcinog*. 2002;33:16–24.
29. Kasraee B, Fallahi MR, Ardekani GS, et al. Retinoic acid synergistically enhances the melanocytotoxic and depigmenting effects of monobenzylether of hydroquinone in black guinea pig skin. *Exp Dermatol*. 2006;15:509–514.
30. Kwarteng A, Ahuno ST, Akoto FO. Killing filarial nematode parasites: role of treatment options and host immune response. *Infectious Diseases of Poverty*. 2016;5:86.
31. Sani BP, Vaid A. Specific interaction of ivermectin with retinol-binding protein from filarial parasites. *Biochem J*. 1988;249:929–932.
32. Lal PG, James ER. Onchocerca retinol- and ivermectin-binding protein activity. *Parasitology*. 1996;112:221–225.
33. Collins MD, Mao GE. Teratology of retinoids. *Annu Rev Pharmacol Toxicol*. 1999;39:399–430.
34. Ross AC, Zolfaghari R. Cytochrome P450s in the regulation of cellular retinoic acid metabolism. *Annu Rev Nutr*. 2011;31:65–87.
35. Wyss R. Chromatographic and electrophoretic analysis of biomedically important retinoids. *J Chromatogr B Biomed Appl*. 1995;671:381–425.