Protection From Arthritis and Myositis in a Mouse Model of Acute Chikungunya Virus Disease by Bindarit, an Inhibitor of Monocyte Chemotactic Protein-1 Synthesis

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Chikungunya virus (CHIKV) is associated with outbreaks of infectious rheumatic disease in humans. Using a mouse model of CHIKV arthritis and myositis, we show that tumor necrosis factor–α, interferon-γ, and monocyte chemotactic protein 1 (MCP-1) were dramatically induced in tissues from infected mice. The same factors were detected in the serum of patients with CHIKV-induced polyarthralgia and polyarthritis, with MCP-1 levels being particularly elevated. Bindarit (MCP inhibitor) treatment ameliorated CHIKV disease in mice. Histological analysis of muscle and joint tissues showed a reduction in inflammatory infiltrate in infected mice treated with bindarit. These results suggest that bindarit may be useful in treating CHIKV-induced arthritides in humans.

Alphaviruses can cause significant inflammatory pathologies, ranging from arthritis to encephalitis. Emerging alphaviruses such as Chikungunya virus (CHIKV), Mayaro virus, and O'nyong-nyong virus are considered significant public health threats by the World Health Organization, with severe cases of debilitating arthritis and arthralgia being reported from a wide range of geographic locations [1].

CHIKV is a positive-sense alphavirus endemic to Africa, India, and Southeast Asia [2]. Infection with CHIKV typically leads to a human disease syndrome involving myalgia, arthritis, arthralgia, rash, and severe lethargy [3]. In a substantial number of patients, symptoms can persist or recur for months or even years following initial infection [4]. In recent CHIKV epidemics, there were a small number of deaths involving neonates and the elderly, or adults with underlying medical conditions [5].

In recent years there have been a number of major outbreaks of CHIKV infection. For example, approximately 250 000 people in Réunion were infected with CHIKV during an epidemic in 2005–2006, and subsequently an additional 3 million individuals were infected during an epidemic in India [2].

The mechanisms by which arthritogenic alphaviruses such as CHIKV cause disease are poorly understood. In a macaque model, Labadie et al first identified macrophages as the main cellular reservoirs for CHIKV infection [6]. Recently, the presence of CHIKV antigen and RNA was shown in synovial macrophages of CHIKV patients [7]. In a chronic case of CHIKV infection, macrophage infiltration of synovium was detected, suggesting that these cells may play an important role in joint inflammation following CHIKV infection [7]. A mouse model of CHIKV disease was recently established by Gardner et al, who proposed that macrophages were essential in CHIKV-induced muscle and ankle joint inflammation [8]. Recent mouse studies provided some evidence for muscle and joint inflammation and associated damage as being the likely cause of CHIKV-induced symptoms in mice [8]. The foot and skeletal muscle were sites of high-level viral replication with tissue inflammatory infiltrates comprising monocytes, macrophages, natural killer cells, and CD4 and CD8 T cells [8]. Studies in the mouse and macaque models have characterized joint and muscle pathology and the relevance of these models to human disease [6, 8]. Similar mouse studies with the related alphavirus Ross River Virus (RRV) have shown that this virus particularly targets the muscle, bone, and joint, leading to macrophage-driven inflammation of these tissues [9]. The importance of macrophages in the mouse model of RRV arthritis was demonstrated by Lidbury et al, who showed that depletion of macrophages using the macrophage-specific inhibitor clodronate significantly reduced joint and muscle inflammation [10].

Current treatment for CHIKV infection is symptomatic, involving analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs), which provide partial relief from rheumatic symptoms [1]. There is an urgent need for the development of improved therapies for infection with CHIKV, particularly because no licensed vaccine is available.

In this study, we tested the efficacy of a small molecule anti-inflammatory drug, bindarit, in the mouse model of CHIKV disease. Bindarit functions largely by inhibiting production of monocyte chemotactic proteins (MCPs) [11], which are key regulators of macrophage migration into sites of inflammation. Mice treated with bindarit were protected from inflammation of joint and skeletal muscle tissues following CHIKV infection. This is the first study to report the critical role of MCP-1 in CHIKV-induced arthritis and myositis and highlights the therapeutic potential of MCP inhibitors in CHIKV disease.

MATERIALS AND METHODS

Patient Serum Samples
Paired acute serum samples from patients presenting with febrile illness, polyarthralgia, and polyarthritis suspected of having CHIKV infection were tested by CHIKV serology at the Ministry of Public Health (MOPH), Thailand. CHIKV infection was defined as a 4-fold rise in hemagglutination inhibition antibody titers in acute and convalescent sera. Twenty-five serum samples from patients with positive CHIKV serology were selected for measurement of cytokines by enzyme-linked immunosorbent assay (ELISA; R&D Systems). Serum from 10 healthy individuals with negative CHIKV serology was also analyzed. Studies were approved by MOPH Thailand.

Mouse Infection
Twenty-four-day-old C57BL/6 mice were inoculated subcutaneously with 10^4 plaque-forming units (PFU) of CHIKV (Réunion isolate passaged twice in C6/36 cells) or RRV. Control animals were injected with diluent only. Mice were scored for disease symptoms every day for 20 days. Mice were sacrificed by CO_2 asphyxiation, and the serum, quadriceps muscles, and ankle joints were removed for cytokine measurement by ELISA (R&D Systems) and determination of virus titers as described previously [8, 9]. Quadriceps muscles and ankle joints were also collected for histological analysis as described previously [10]. Mice were held and manipulated in accordance with institutional guidelines that are in compliance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH publication number 85-23, 1985). The experimental protocols were approved by the University of Canberra and Angelini Research Center research committees.

Bindarit Treatment of Mice
Bindarit, 2-methyl-2-[[1-(phenylmethyl)-1H-indazol-3-yl] methoxy] propanoic acid (Angelini Research Center, Rome, Italy) was given intraperitoneally to mice (100 mg/kg) or vehicle (0.5% methylcellulose, Sigma) twice a day from day of infection for 5 days.

Statistical Analysis
Mouse cytokine analyses were carried out using the Student unpaired t test. Data for mouse disease scores and human cytokines were analyzed using Mann–Whitney U test. Data were considered statistically significant at $P < .05$.

RESULTS

High Levels of Proinflammatory Cytokines Are Detected in CHIKV-Infected Humans and Mice
Levels of proinflammatory cytokines were measured in serum from patients suffering from acute CHIKV-induced polyarthralgia and polyarthritis. Samples from CHIKV-infected individuals were collected during the acute phase of the disease, on average 4 (SD 5.8) days after the onset of the disease. Serum from patients with acute CHIKV-induced polyarthralgia and polyarthritis had significantly higher levels of MCP-1 ($P = .0015$; Figure 1A), tumor necrosis factor (TNF)–α ($P = .0285$; Figure 1B), and interferon (IFN)–γ ($P = .0263$; Figure 1C) compared with serum from healthy individuals. The average platelet count was 236 588 cells/μl, ranging from 115 000 to 316 000 cells/μl. There were no correlations between platelet counts and the levels of MCP-1, IFN-γ, or TNF-α. In addition, MCP-1, TNF-α, and IFN-γ were measured in serum and homogenized skeletal muscle and ankle joint tissues of alphavirus-infected mice. The amount of MCP-1, TNF-α, and IFN-γ proteins produced by infected mice was greater than that in uninfected control mice (Figure 1D–F). These findings are consistent with the prominent increase in MCP-1 and IFN-γ and, to a lesser extent, TNF-α that was reported in a primate model of CHIKV infection [6].

Bindarit Treatment Ameliorated CHIKV-Induced Disease
Since MCP-1 levels were detected at high levels in patients and mice with CHIKV disease, we tested the therapeutic potential of bindarit, an inhibitor of MCP-1 synthesis, in CHIKV disease in mice. Bindarit-treated or untreated mice were infected with 10^4 PFU of CHIKV and animals were monitored for the development of disease signs. Infection of untreated mice resulted in severe disease with peak clinical scores observed on day 10 after infection (Figure 2A). In contrast, disease signs in bindarit-treated mice were mild and of much shorter duration (Figure 2A).

We tested whether differences in viral titers in the ankle joint or skeletal muscle could explain the reduced severity of disease observed following bindarit treatment of CHIKV-infected mice. Analysis showed no significant differences in virus titers in bindarit-treated CHIKV-infected mice compared with untreated infected mice at all time points after infection (not shown).
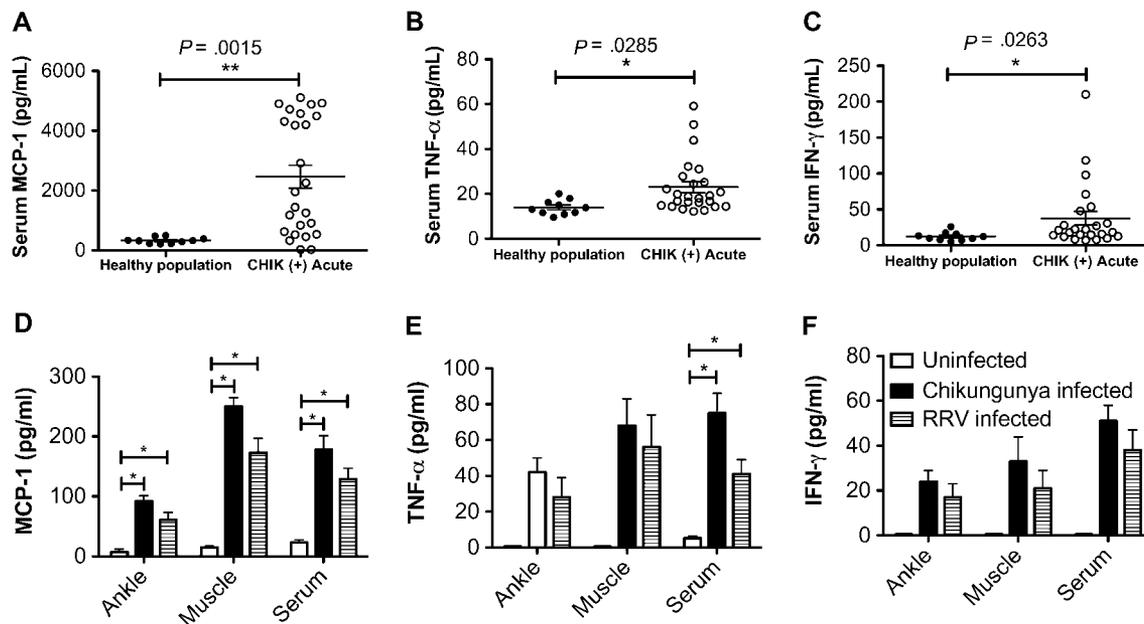
Figure 1. Proinflammatory cytokines are upregulated in the serum of patients with acute Chikungunya (CHIK) infection and in the tissues of alphavirus-infected mice. (*A–C*) Acute serum samples from patients presenting with febrile illness and polyarthritis (25 positive for CHIK) and 10 healthy controls were depersonalized and the levels of monocyte chemotactic protein (MCP)–1, tumor necrosis factor (TNF)–α, and interferon (IFN)–γ were measured by enzyme-linked immunosorbent assay (ELISA). Each point represents a single patient sample. Significant differences are marked by an asterisk. (*D–F*) C57BL/6 mice were infected subcutaneously with 10^4 plaque-forming units of CHIK or Ross River Virus or mock-infected with diluent alone. At day 10 after infection, mice were sacrificed and serum, ankle joint, and quadriceps muscle tissues were collected. Ankle joint and quadriceps muscle tissues were homogenized in phosphate-buffered saline. Cell debris was pelleted following centrifugation and the supernatant was collected and stored at −80°C. Protein levels of MCP-1, TNF-α, and IFN-γ in tissues of virus-infected mice (*n* = 4) and mock-infected mice (*n* = 4) were determined by ELISA (performed in duplicate).

To determine the effects of bindarit treatment on tissue inflammation and pathology, hind limb joint and skeletal muscle tissues were observed by histology. Inflammation was abolished in synovial tissue and skeletal muscle in bindarit-treated mice infected with CHIKV (Figure 2*B*, ii, iv, vi). In contrast, severe inflammation was observed in synovial tissue and skeletal muscle of the infected control group at 10 days after infection. (Figure 2*B*, i, iii, v). Extensive disruption of striated muscle fibers was evident in infected untreated mice, while muscle tissue from infected bindarit-treated mice exhibited no detectable damage.

DISCUSSION

In this paper we demonstrated that (1) high levels of proinflammatory cytokines, namely MCP-1 and TNF-α, are produced following CHIKV infection in humans and mice and (2) the MCP-1 synthesis inhibitor bindarit can ameliorate disease symptoms and tissue damage associated with CHIKV infection in mice. These results highlight the potential for anti-inflammatory strategies that target macrophage migration for the treatment of CHIKV disease.

The recently established mouse model of CHIKV disease recapitulates many aspects of the human disease and serves as an accurate model to study disease pathogenesis and new treatment options. At present, alphavirus-induced arthritic diseases are mainly treated with NSAIDs, which provide some symptomatic relief but do not significantly modify the underlying disease processes. Bindarit treatment markedly reduced disease symptoms and tissue damage associated with CHIKV infection. A recent study suggested that inflammatory cells cause the musculoskeletal symptoms and tissue damage in CHIKV-infected mice [8], and our results support this conclusion by showing a correlation between the therapeutic effects of bindarit and its ability to reduce the influx of inflammatory cells into muscle and joint tissue. Bindarit treatment led to reduced expression of MCP-1 and TNF-α in muscle and joint tissues in CHIKV-infected mice (not shown), consistent with the recently described ability of bindarit to inhibit production of these cytokines in RRV-infected mice [12]. The reduction in MCP-1 production contributed to the decreased macrophage infiltration into tissues (not shown), while lower levels of TNF-α, one of the several proinflammatory products of macrophages, are also likely to contribute to the reduced tissue destruction observed in bindarit-treated mice. Furthermore, bindarit treatment commencing on day 5 after infection (established disease) can prevent the development of severe disease, suggesting that the administration of bindarit at the onset of symptoms can have a therapeutic effect (not shown).

The inhibition of cytokines with antibodies or small molecule inhibitors promises benefits in a range of inflammatory
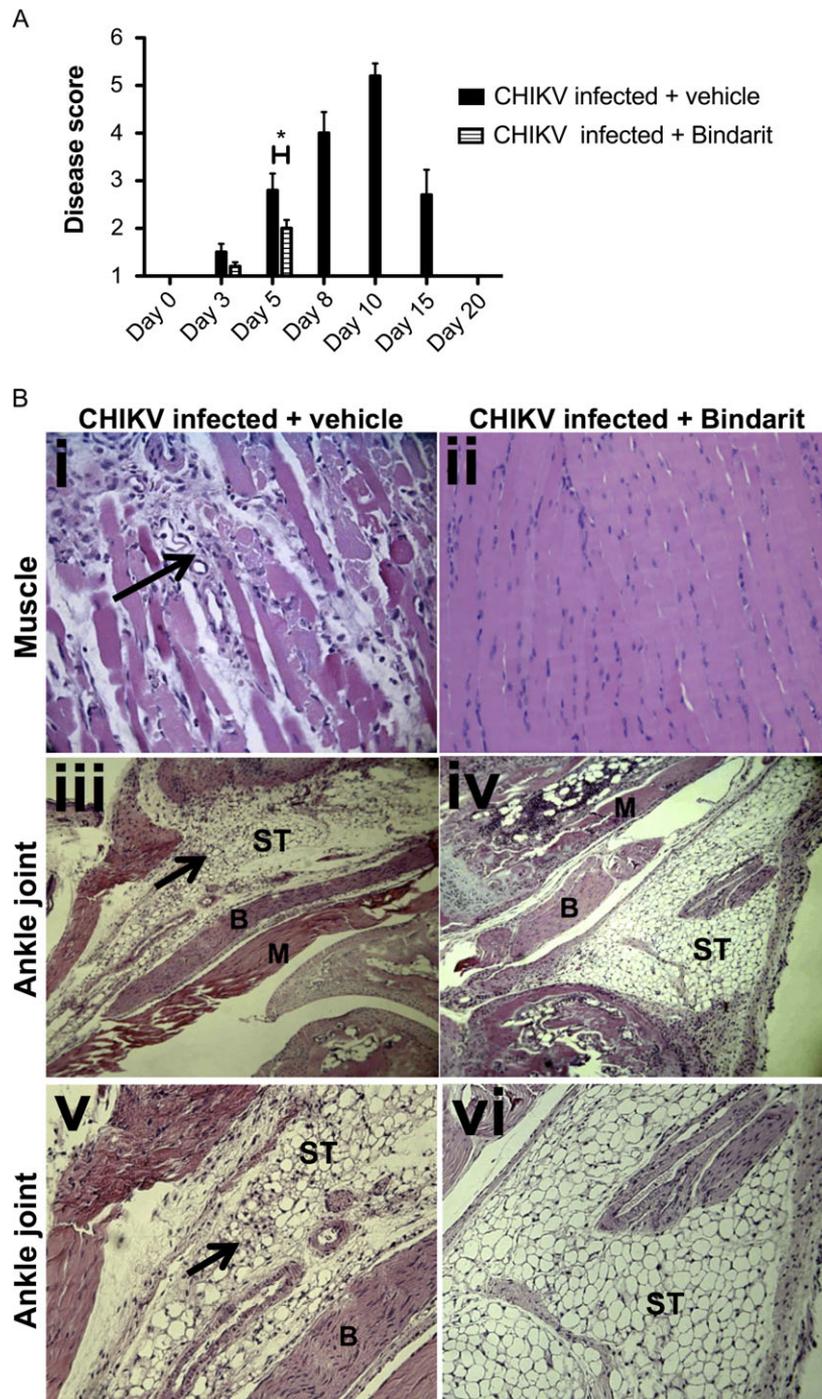
Figure 2. Bindarit treatment ameliorates Chikungunya (CHIKV) disease in mice. Mice were infected subcutaneously with 10^4 plaque-forming units of CHIKV. Mock-infected mice were injected with phosphate-buffered saline alone. Mice received peritoneal injections of bindarit or the corresponding vehicle twice a day from day of infection for 5 days. *A*, Disease signs were determined by assessing grip strength and altered gait based on the following scales: 0, no disease; 1, ruffled fur; 2, very mild hind limb weakness; 3, mild hind limb weakness; 4, moderate hind limb weakness; 5, severe hind limb weakness/dragging; 6, complete loss of hind limb function; 7, moribund; 8, death. Each data point represents the mean $\pm$ SD for 7 mice per group. Significant differences are marked by an asterisk. *B*, At 10 days after infection, mice were perfused with 4% paraformaldehyde and 5-µm-thick paraffin-embedded sections from quadriceps muscle were stained with hematoxylin and eosin (H&E); (i) CHIKV-infected, vehicle-treated mice, (ii) CHIKV-infected, bindarit-treated mice. Following decalcification, 5-µm-thick, paraffin-embedded sections generated from ankle joints were stained with H&E; (iii and v) CHIKV-infected, vehicle-treated mice and (iv and vi) CHIKV-infected, bindarit-treated mice. B, bone; M, muscle; P, periosteum; ST, synovial tissue. The images are representative of 4 mice per group. Magnification: i, ii (400×); iii, iv (100×); v, vi (200×).

diseases [13]. The most successful therapeutic cytokine inhibitors are biologics developed to inhibit the function of TNF-α, which have been very successful in treating numerous inflammatory diseases, particularly rheumatoid arthritis. However, concerns have been raised about the safety profile of these drugs, with a minority of patients suffering from severe infections, presumably due to an impaired inflammatory response [14]. Indeed, we found that treatment of RRV-infected mice with the TNF inhibitor etanercept resulted in a marked exacerbation of arthritis and myositis and increased viral load [15]. The efficacy and low toxicity of bindarit in several animal models of inflammatory disease demonstrate that small molecule inhibitors of the cytokine/chemokine system should be seriously considered as potential therapeutics for arthritis resulting from infection with CHIKV.

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