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ORIGINAL ARTICLE

Diminazene aceturate (Berenil) downregulates *Trypanosoma congolense*-induced proinflammatory cytokine production by altering phosphorylation of MAPK and STAT proteins

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Abstract
Diminazene aceturate (Berenil) is the most commonly used trypanolytic agent in livestock. We previously showed that Berenil downregulates *Trypanosoma congolense* (*T. congolense*)-induced cytokine production in macrophages both in vitro and in vivo. Here, we investigated the molecular mechanisms through which the drug alters *T. congolense*-induced cytokine production in macrophages. We show that pretreatment of macrophages with Berenil significantly downregulated *T. congolense*-induced phosphorylation of mitogen-activated protein kinase (p38), signal transducer and activator of transcription (STAT) proteins including STAT1 and STAT3, and NFκB activity both in vitro and in vivo. Collectively, our results reveal a mechanistic insight through which Berenil downregulates *T. congolense*-induced cytokine production in macrophages by inhibiting key signaling molecules and pathways associated with proinflammatory cytokine production.

Keywords Diminazene aceturate (Berenil) · Trypanosome · Macrophages · MAPK · STAT1 · Proinflammatory cytokines

Introduction

African trypanosomes are extracellular protozoan parasites that cause disease in both humans and animals. According to the World Health Organization (WHO), Human African Trypanosomiasis (HAT) is a major threat to the health of over 60 million people in 36 countries in Sub-Saharan Africa [1]. Although the disease causes significant human mortality and morbidity in many underdeveloped regions of Africa, its major economic impact relates to its adverse effect on livestock production and farming in the affected areas. It is estimated that in Sub-Saharan African countries alone, the annual cattle production loss due to the disease is about $12 billion [2]. Thus, the disease contributes negatively to food and economic security. Several species of African trypanosomes including *Trypanosoma* (*T.*) *congolense*, *T. vivax*, and *T. brucei brucei* cause disease in animals. Among these, *T. congolense* is the most important pathogen for livestock in Sub-Saharan Africa.

Macrophages play a crucial role in the control of many protozoan parasitic infections including African trypanosomes. Intact monocytic cell system is important for the initiation and maintenance of anti-trypanosome responses [3]. Mononuclear phagocytic system plays a crucial role in the phagocytosis of opsonized trypanosomes, which is the major mechanism for clearance of trypanosomes from the blood stream [4, 5]. During the course of trypanosome infection, the numbers of macrophages significantly increase in many organs including the liver, spleen, and lymph nodes and these cells display morphological and functional features of activation [6, 7]. In addition to their role in phagocytosis and clearance of parasites, activated macrophages also produce proinflammatory cytokines and thus play important role in mediating immunity and immunopathology in infected mice. The activation of macrophages during trypanosome infection is due in part to their exposure to parasite components and host-derived IFN-γ, which is produced in response to parasite antigens [8].

Experimental infection with African trypanosomes is associated with profound enlargement of the spleen and liver. In experimental *T. brucei* infection, a large percentage of cells in the enlarged spleen exhibit membrane and functional

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characteristics associated with activated macrophages. These activated macrophages have been shown to release IL-12, TNF, and nitric oxide during the first 2 weeks of infection and this is associated with modulation of host immunity and resistance [9]. In *T. congolense* infection, complement and antibody-mediated phagocytosis by splenic and liver (kupffer cells) macrophages is one of the primary mechanisms by which trypanosomes are cleared from an infected host [4, 5]. Parasites coated with antibodies including IgM and IgG are taken up by macrophages resulting in their activation and production of proinflammatory cytokines [10]. In addition, activated macrophages present trypanosomal antigens to T cells in an MHC class II–dependent manner resulting in the production of IFNs [11]. This leads to further activation of macrophages leading to enhanced proinflammatory cytokine production. During peak parasitemia, there is massive phagocytosis of trypanosomes leading to hyper-activation of macrophages and increased production of monokines and IFN-γ [12]. Death of trypanosome-infected animals is attributed to cytokine over production by macrophages and T cells leading to systemic inflammatory response-like syndrome [12–14].

Berenil (Diminazene aceturate) has been used for the treatment of animal trypanosomiasis for over 60 years and is the most commonly used therapeutic agent for trypanosomiasis in livestock. Despite this large-scale use, its mechanism of action is not fully understood. A complete understanding of its molecular mechanisms of action is critical in order to maximize its therapeutic and prophylactic potentials. Previously, we showed that Berenil treatment causes significantly reduction in serum levels of proinflammatory cytokines and alters the activation status of lymphocytes in spleens and livers of *T. congolense*-infected mice [15]. In addition, we further showed that Berenil also downregulates LPS-induced proinflammatory cytokine (IL-1β, IL-6, IL-12, and TNF-α) production by macrophages [16]. In this study, we investigated whether Berenil has any effect on *T. congolense*-induced signaling pathways in macrophages. We show that pretreatment of macrophages with Berenil significantly downregulated *T. congolense*-induced phosphorylation of ERK, p38, STAT1, and STAT3 leading to suppression of proinflammatory cytokine production including IL-1β, IL-6, IL-12, and TNF-α.

Materials and methods

Mice

Six- to 8-week-old female C57BL/6 and CD1 mice were purchased from Charles River Laboratory, Quebec, or from the University of Manitoba Central Animal Care Services (CACS) breeding facility. All mice were housed in specific pathogen-free environment at CACS.

Preparation of trypanosomal whole cell extract

T. congolense (Trans Mara Strain), variant antigenic type (VAT) TC13, was used in this study [17]. Frozen TC13 stabilates were expanded in immunosuppressed CD1 mice as previously described [17]. Blood was collected from infected CD1 mice on day 3 post-infection by cardiac puncture and parasites were purified from blood using DEAE-cellulose anion-exchange chromatography [18]. The isolated parasites were washed and resuspended in Tris-saline glucose (TSG) at a final concentration of 10^8/ml and subjected to 3–5 cycles of sonication (5 min per cycle). The sonicates were further subjected to 8 cycles (30 min/cycle) of freeze/thawing at − 80 °C, aliquoted, and stored at − 80 °C until used. Endotoxin level in whole cell extract (WCE) preparations was determined using the LAL kit (E-TOXATE, Sigma) and was less than 0.05 EU/ml.

Immortalized cell line and in vitro cell culture

The murine macrophage cell lines (ANA-1 cells) used in this study are retrovirus immortalized bone marrow–derived macrophage cell lines from the relatively resistant C57BL/6 mice [19]. ANA-1 cells were cultured in complete RPMI-10 medium (RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml of penicillin, and 100 μg/ml of streptomycin). Primary bone marrow–derived macrophages from C57BL/6 mice were generated as previously described [20]. Briefly, bone marrow cells were isolated from femur and tibia of C57BL/6 mice and differentiated into macrophages using conditioned media (complete RPMI-10 medium supplemented with 30% L929 cell culture supernatant). The cells were harvested on the 7th day, washed, resuspended in complete medium, and used for experiments.

Western blot

Phosphorylation of MAPKs, STATs, and NFκB p65 subunit was determined by western blot. ANA-1 cells and BMDMs were seeded into petri-plates and serum starved for 24 h. These cells were then treated with Berenil (10 μg/ml) over night (ON) and then stimulated with *T. congolense* whole cell lysate (WCE) for different time points as indicated. The cells were washed twice with ice cold PBS and lysed with NP40 lysis buffer supplemented with a protease inhibitor cocktail, 1 mM sodium orthovanadate and 1 mM phenylmethylsulfonyl fluoride. Total protein in the lysate was determined using the BCA protein assay kit (Thermo Scientific) according to the manufacturer's suggested protocol. The lysates (10 μg/sample) were resolved in 10% SDS-PAGE, transferred onto polyvinylidene difluoride (PVDF) membranes (Amersham Biosciences, Quebec, Canada), and blocked with 5% BSA in Tris-buffered saline with tween 20 (TBST) for 2 h at room temperature. The membranes were incubated overnight at room temperature with polyclonal rabbit anti-mouse antibodies against phosphorylated p38, ERK1/2, JNK, STAT1, STAT3, and NFκB p65. Thereafter, the blots were washed five times with TBST and incubated with mouse anti-rabbit HRP-conjugated secondary antibody at room temperature for 30 min, and the bands were revealed using enhanced chemiluminescence (ECL) reagents (Amersham, GE Healthcare Biosciences, Pittsburgh, PA). The blots were routinely stripped and reprobed with antibodies specific for total p38, ERK, JNK, STAT1, and STAT3 that were used as loading controls.

Isolation of peritoneal macrophages

Mice were treated with Berenil (14 mg/kg) overnight and then inoculated intraperitoneally with or without trypanosome whole cell lysate (10^8/ml, 100 μl/mouse) or 10^3 live parasites. The mice were sacrificed at different time points and the peritoneal lavage fluid was collected as previously explained [21]. Macrophages were isolated from peritoneal lavage fluids, lysed with NP40 lysis buffer and the lysates used for western blot as explained above.

Cytokine ELISA

BMDMs and ANA cells were pretreated overnight with Berenil and stimulated with WCE for 12–24 h. Thereafter, the supernatant fluids were collected and the concentrations of IL-1β, IL-6, IL-12, and TNF-α were determined by ELISA using paired antibodies and appropriate cytokine standard (eBioscience, San Diego, CA) according to the manufacturer's suggested protocols.

Quantification of phosphorylated and total NFκB p65

Phosphorylation of NFκB p65 was confirmed by Fast activated Cell-based ELISA NFκB p65 profiler kit (Active Motif, Carlsbad, CA) according to the manufacture's protocol. Briefly, BMDMs were cultured in 96-well plates, treated overnight with Berenil 10 μg/ml, and then stimulated with *T. congolense* WCE. At different times, the cells were fixed rapidly, washed, and incubated overnight at 4 °C with phospho and total NFκB p65 primary antibody. The plates were washed and incubated with HRP-conjugated secondary antibody and developed. The relative number of cells in each well was determined by using crystal violet. Both phosphorylated and total NFκB p65 signals were normalized to corresponding cell numbers and the ratios of phosphorylated to total NFκB p65 over controls were determined.

Luciferase reporter constructs and transient transfection

ANA-1 cells were seeded into 24 well plates and at 70–80% confluency, they were transiently (for 24 h) transfected by using Turbofect (Fermentas Canada, Ottawa, Canada) with 0.8 μg/ml IL-6 promoter luciferase DNA vector and 0.2 μg/ml Renilla luciferase vector pRL-TK according to the manufacturer's protocol. The cells were then washed and treated with Berenil and WCE for 12 h. After additional washing, the cells were lysed with lysis buffer and the cell lysates were collected. Using Dual Luciferase Assay system kit (Promega, Madison, WI), luciferase activity was assessed by a luminometer. The values were normalized to Renilla luciferase activity and expressed relative to transfected unstimulated control cells. The IL-6 promoter luciferase constructs used in this study were a gift from Dr. Gail Bishop, University of Iowa.

Statistical analysis

Cytokine data are presented as mean ± SEM. Two-tailed Student's *t* test and two-way ANOVA were used to compare differences in cytokine production. Differences were considered significance if $p < 0.05$. All analyses were carried out using GraphPad Prism software.

Results

Berenil pretreatment downregulates *T. congolense*-induced IL-6 and IL-12 production in macrophages We previously showed that Berenil treatment dramatically lowers serum proinflammatory cytokine levels in *T. congolense*-infected mice and that this was suspected to be due to direct effect of the compound on CD11b$^+$ cells in the spleens and livers of infected mice [15]. Indeed, we confirmed that trypanosomes induce cytokine (IL-6 and IL-12) production in macrophage cell lines (ANA-1) and bone marrow–derived macrophages (BMDMs, Fig. 1A–D). To directly determine the impact of Berenil *T. congolense*-induced cytokine production in macrophages, we pretreated immortalized macrophage cell lines (ANA-1) and primary macrophages (BMDMs) with Berenil overnight, stimulated them with *T. congolense* whole cell extract (WCE) for 14–16 h, and assessed cytokine production by ELISA. The concentration of Berenil used in the present studies have been previously shown to be non-toxic to the cells as assessed by MTT, trypan blue dye exclusion, and propidium iodide assays [16]. Berenil treatment suppressed *T. congolense*-induced production of proinflammatory cytokines (including IL-1β, IL-6, IL-12p40, and TNF-α) by both ANA-1 cells (Fig. 1A–D) and BMDMs (Fig. 1E–H). These results indicate that as observed in vivo [15], Berenil pretreatment directly downregulates

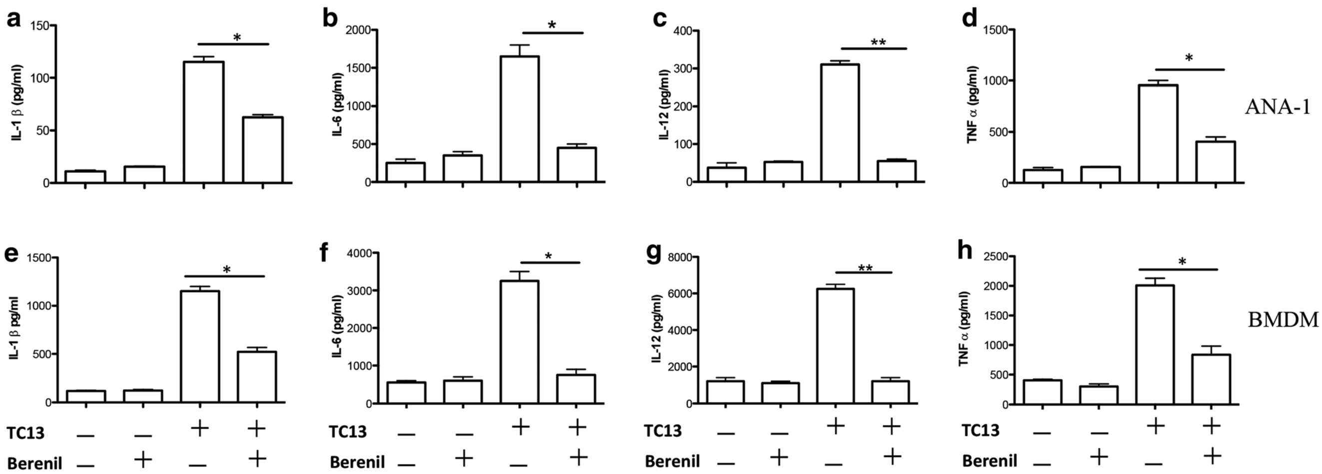

Fig. 1 Berenil pretreatment downregulates *Trypanosoma congolense* (TC13)-induced cytokine production in macrophage cell lines (ANA) and bone marrow–derived macrophages (BMDM). ANA cells and BMDMs were treated with Berenil (10 μg/ml) overnight and inoculated with TC13 lysate (1:10 macrophage:parasite equivalent ratio). After 14–16 h, the culture supernatant fluids were collected and the levels of IL-1β, IL-6, IL-12, and TNF-α were measured by sandwich ELISA. Shown are IL-1β, IL-6, IL-12, and TNF-α production by ANA cells (A–D) and BMDMs (E–H). The data presented are representative of three different experiments with similar results. *$p < 0.05$; **$p < 0.01$

T. congolense-induced proinflammatory cytokine production in macrophages in vitro.

Berenil pretreatment downregulates *T. congolense*-induced MAPK and STAT phosphorylation in macrophages MAPKs and STATs are important signaling proteins that regulate proinflammatory cytokine production in immune cells [22]. Recent reports show that *Trypanosoma cruzi*- and *Toxoplasma gondii*-mediated induction of proinflammatory cytokine production in macrophages is dependent on MAPK, STAT1, and STAT3 phosphorylation [23, 24]. We recently showed that Berenil inhibits LPS-induced proinflammatory cytokine production in macrophages via suppressing MAPK and STAT phosphorylation events [15]. Therefore, we investigated whether inhibition of *T. congolense*-induced proinflammatory cytokine production in macrophages following Berenil treatment is mediated by its effect on MAPKs and STATs phosphorylation. We pretreated ANA cells with Berenil, stimulated them with WCE and assessed cellular lysates at various time points for phosphorylation of inhibited ERK, p38, STAT1, and STAT3 by western blot. We found that while pretreatment with Berenil did not alter WCE-induced ERK phosphorylation, it dramatically inhibited p38, STAT1, and STAT3 phosphorylation in ANA cells (Fig. 2A–D), suggesting that the inhibitory effect of Berenil on cytokine production may be mediated by its downregulation of these key signaling molecules.

To determine whether the inhibitory effect of Berenil on MAPK and STAT phosphorylation observed with immortalized ANA-1 cells is reproducible in primary macrophages, we pretreated BMDMs with Berenil, stimulated them with WCE, and performed western blot from lysates at different times to assess MAPK and STAT phosphorylation. In line with ANA-1 cells, Berenil treatment did not alter ERK phosphorylation but downregulated the p38, STAT1, and STAT3 phosphorylation in BMDMs (Fig. 3A–D), suggesting that the effects observed in ANA-1 cells were not related to the immortalization process. Collectively, these results show that Berenil inhibits phosphorylation of p38, STAT1, and STAT3 in macrophages and this could be responsible for the downregulation of *T. congolense*-induced proinflammatory cytokines observed in treated cells.

Berenil pretreatment downregulates *T. congolense*-induced MAPK and STAT phosphorylation in peritoneal macrophages Next, we evaluated whether the effect of Berenil on *T. congolense*-induced MAPK and STAT phosphorylation is reproducible in vivo. We pretreated mice with Berenil (14 mg/kg) overnight, injected them with WCE, and sacrificed them at different time points to assess phosphorylation of ERK, p38, STAT1, and STAT3 in peritoneal macrophages directly ex vivo. Consistent with in vitro findings, Berenil treatment significantly downregulated WCE-induced phosphorylation of p38, STAT1, and STAT3, but did not affect ERK phosphorylation (Fig. 4A–D). This was associated with significant reduction in IL-6 and IL-12 levels in the peritoneal lavage fluids (Fig. 4E, F). Collectively, these results further confirm that Berenil downregulates *T. congolense*-induced proinflammatory cytokine production by altering phosphorylation of MAPKs and STATs in vivo.

Berenil downregulates *T. congolense*-induced phosphorylation of NFκB p65 and IL-6 promoter activity Nuclear factor-κB (NFκB) family of transcription factors plays critical roles in cytokine production, inflammation, cell proliferation, and survival. The most activated form of NFκB is a heterodimer

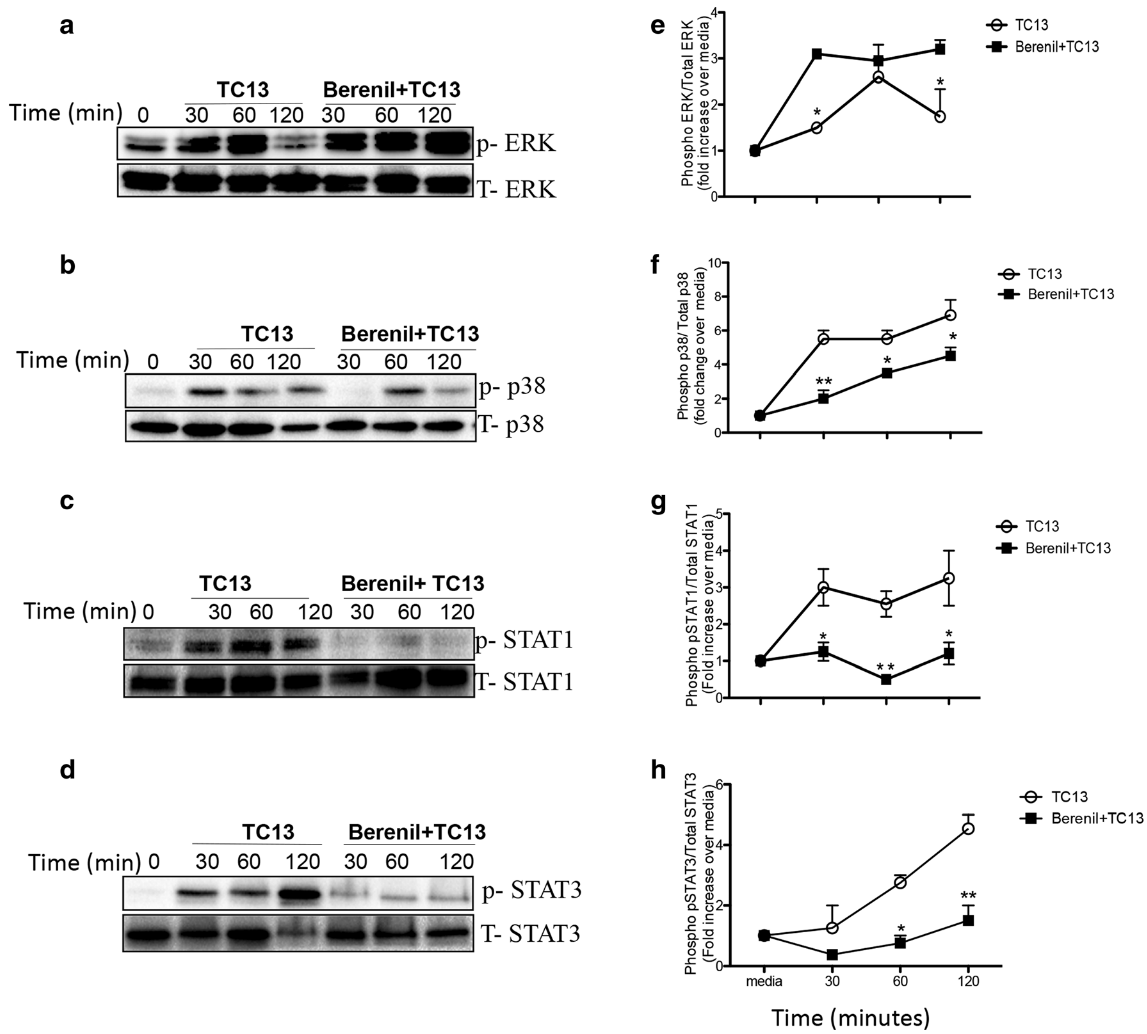

Fig. 2 Berenil alters TC13-induced MAPK and STAT phosphorylation in ANA-1 cells. ANA-1 cells were treated with Berenil (10 μg/ml) overnight and stimulated with TC13 lysate. At the indicated times, the cells were lysed and the lysates were assessed by Western blot for phosphorylation of ERK (A), p38 (B), STAT1 (C), and STAT3 (D). The same blots were stripped and reprobed with Abs against total ERK, p38, STAT1, STAT3, and used as loading controls. The right panel shows the corresponding densitometry (E–H). The western blot results represent one of three independent experiments with similar findings. *$p < 0.05$; **$p < 0.01$

consisting of p65 and p50 subunits and they control genes that regulate a broad range of biological processes including cytokine production, inflammation, and stress responses. Therefore, we evaluated whether Berenil suppression of *T. congolense*-induced proinflammatory cytokine production results from its inhibitory effect on NFκB activation. Pretreatment of BMDMs with Berenil inhibited *T. congolense*-induced phosphorylation of NFκB p65 subunit as assessed by Western blot and ELISA (Fig. 5A, B).

To further determine the mechanism through which Berenil suppresses *T. congolense*-induced cytokine production, we investigated whether Berenil has any direct effect on promoter activity of IL-6 gene. ANA cells were transiently transfected with IL-6 luciferase reporter constructs, pretreated overnight with Berenil, and stimulated with *T. congolense*. ANA cells transfected with IL-6 promoter construct showed a significant increase in response to *T. congolense* stimulation. In contrast, Berenil pretreatment dramatically downregulated *T. congolense*-induced IL-6 promoter activity (Fig. 5C), suggesting that Berenil affects gene transcriptional processes in macrophages leading to downregulation of proinflammatory cytokines in response to *T. congolense*.

Discussion

We recently identified important signaling molecules and pathways involved in *T. congolense*-induced proinflammatory cytokine production in macrophages [15]. In addition, we showed that treatment of *T. congolense*-infected mice with Berenil downregulated proinflammatory cytokine production and this was associated with survival from an otherwise lethal

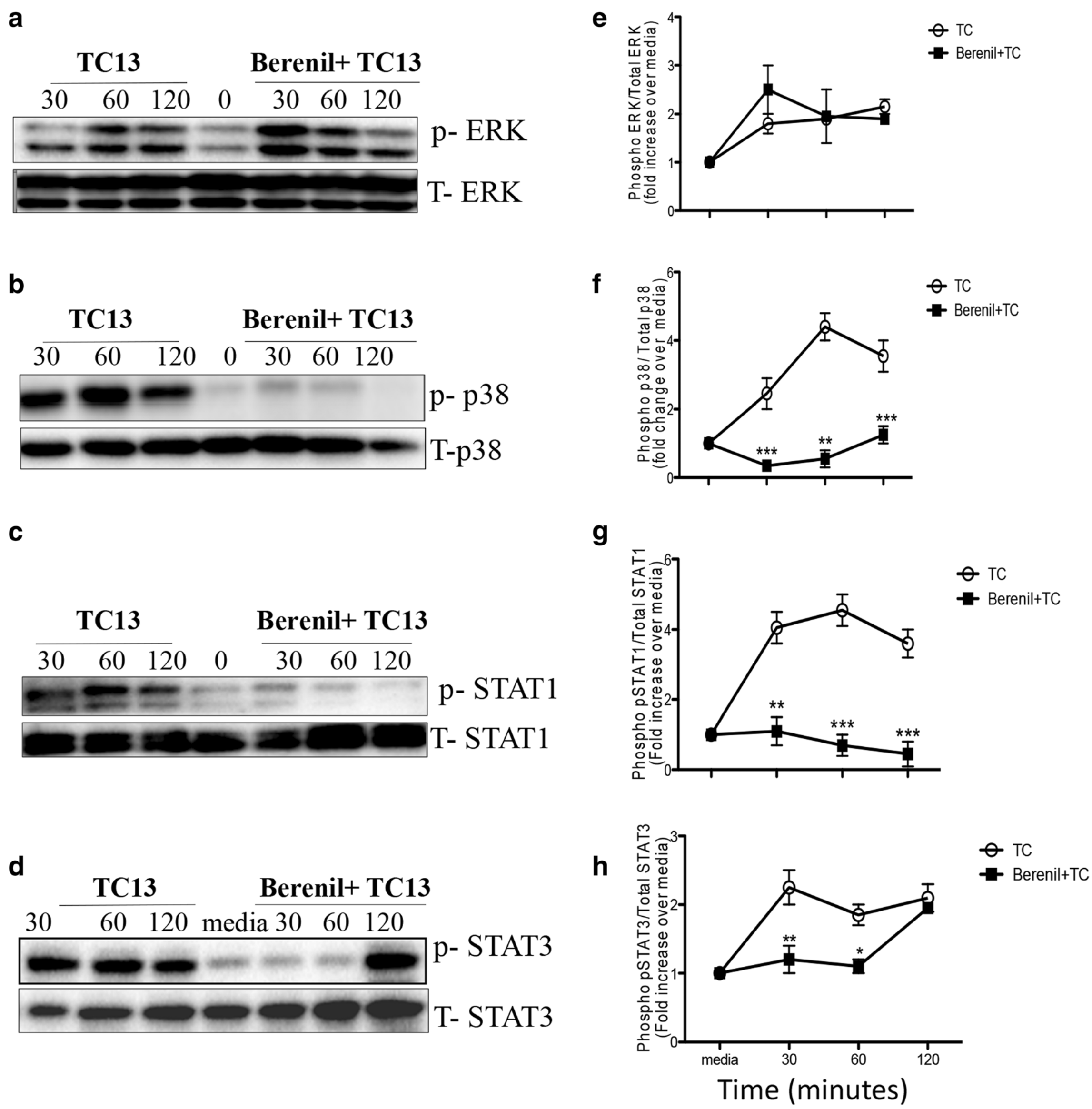

Fig. 3 Berenil downregulates TC13-induced MAPK and STAT phosphorylation in BMDMs. BMDMs were treated with Berenil (10 μg/ml) overnight and stimulated with TC13 lysate. At the indicated times, the cells were lysed and the lysates were assessed by Western blot for phosphorylation of ERK (A), p38 (B), STAT1 (C), and STAT3 (D). The right panel shows the corresponding densitometry (E–H). The same blots were stripped and reprobed with Abs against total ERK, p38, STAT1, and STAT3 and used as loading controls. The western blot results represent one of three independent experiments with similar findings. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

infection [15]. Collectively, these studies present an important question: does Berenil inhibit *T. congolense*-induced proinflammatory cytokine production in macrophages and if it does, is this mediated via inhibition of MAPKs and STATs phosphorylation? The data presented here identify for the first time the effect of Berenil on *T. congolense*-induced intracellular signaling pathways that lead to proinflammatory cytokine production in macrophages. Using both macrophage cell lines and primary BMDMs, we showed that Berenil pretreatment significantly downregulated *T. congolense*-induced proinflammatory cytokines production by these cells. Given that phosphorylation of MAPKs and STAT proteins is critical for the production of these cytokines [25–27], we tested the effect of Berenil on phosphorylation of ERK, p38, STAT1, and STAT3 proteins following *T. congolense* stimulation. Berenil pretreatment did not alter *T. congolense*-induced ERK phosphorylation whereas phosphorylation of p38, STAT1, and STAT3 proteins was significantly downregulated. This suggests that the inhibitory effect of Berenil is not global and that its action is selective in the signal transduction pathways that it affects. We further confirmed these in vitro observations by showing that Berenil treatment downregulated the p38, STAT1, and STAT3 phosphorylation in peritoneal macrophages following in vivo (intraperitoneal) parasite injection.

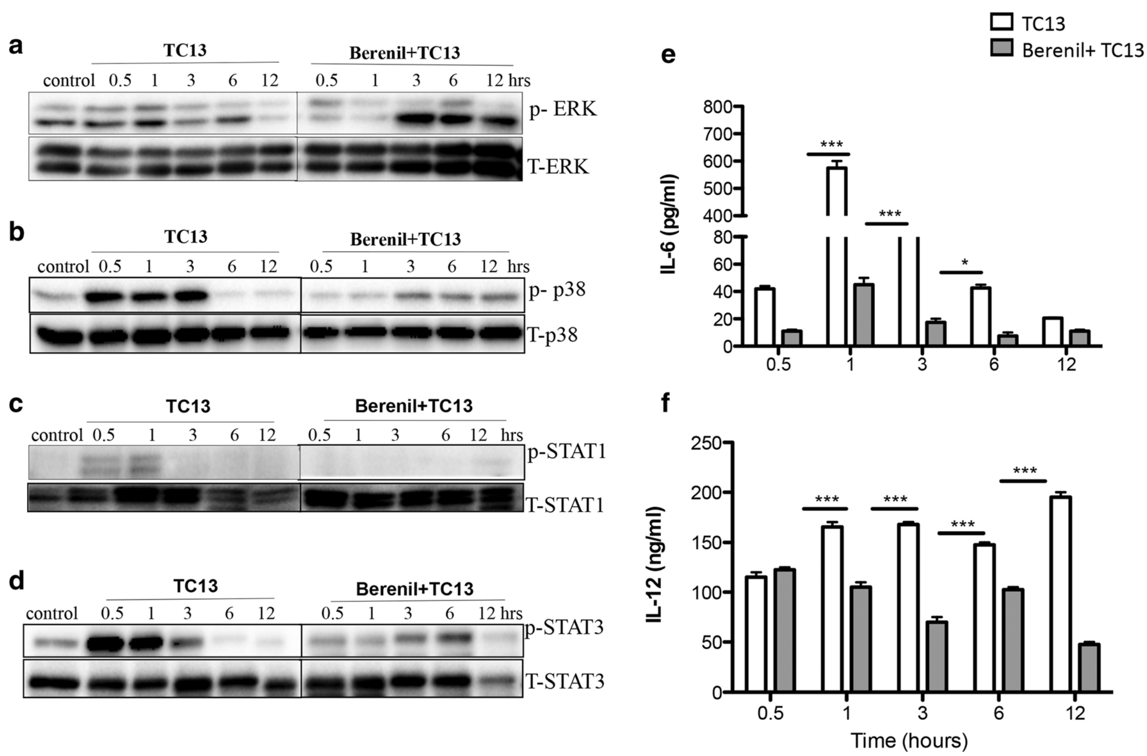

Fig. 4 Berenil downregulates *T. congolense*-induced MAPK and STAT phosphorylation in vivo. Groups of C57BL/6 mice were treated with Berenil overnight and then inoculated with TC13 lysate (containing 10^6 parasites). At indicated times, mice were sacrificed and lysates of peritoneal macrophages were assessed for phosphorylation of ERK, p38, STAT1, and STAT3 (A–D) by western blot. In addition, IL-6 and IL-12 levels in the peritoneal lavage fluids were determined by ELISA (E, F). The data presented are representative of two independent experiments with similar results ($n = 4$ mice per each time point). $*p < 0.05$; $***p < 0.001$

This was associated with a concomitant suppression of proinflammatory cytokines resulting in significantly lower levels of IL-6 and IL-12 in peritoneal lavage fluids.

NFκB is a crucial transcription factor for proinflammatory cytokine production by innate immune cells, and several protozoan parasites including *T. cruzi* have been shown to trigger NFκB activation through Toll-like receptor-2 [28]. GPI molecules from *T. cruzi* have been shown to trigger phosphorylation of ERK, p38, and IκB leading to the activation of NFκB and a resultant activation of proinflammatory genes [29, 30]. *Trypanosoma brucei* (a close relative of *T. congolense*) have also been shown to initiate the cascade of ERK, p38, JNK, and NFκB phosphorylation events resulting in the expression of a number of proinflammatory genes including TNF, IL-12, IL-6, and nitric oxide [31]. We showed that *T. congolense* induces the activation of NFκB p65 subunit and that Berenil pretreatment downregulates this activity. Interestingly, we found that Berenil also downregulated *T. congolense*-induced IL-6 promoter activity, suggesting that the compound may directly alter proinflammatory cytokine gene transcription in macrophages.

Although Berenil has been the most commonly used therapeutic agent for treatment of animal trypanosomiasis for over 60 years, its molecular mechanisms of action are not completely known. Our previous report showed that Berenil treatment of *T. congolense*-infected BALB/c mice led to a dramatic reduction in serum levels of IL-6, IL-12, TNF, and MCP-1, and this was as associated with survival from an otherwise lethal infection [15]. However, the exact mechanism through which Berenil treatment blocks proinflammatory cytokine production following *T. congolense* infection was unknown. The work presented here demonstrates for the first time how Berenil might block proinflammatory cytokine production in macrophages from infected mice. They show that Berenil's inhibitory effect on proinflammatory cytokine production is mediated by its effect on signaling events responsible for NFκB-mediated macrophage activation. This includes its inhibitory effect on *T. congolense*-induced p38, STAT1, and STAT3 phosphorylation in macrophages.

Previous studies show that Berenil also inhibits LPS-induced phosphorylation of MAPKs and STAT proteins leading to suppression or proinflammatory cytokine production by macrophages [16]. This observation suggests that Berenil may globally inhibit inflammatory cytokine production by immune cells. Within the pharmaceutical industry, there is an increasing interest in targeting the MAPK and other intracellular pathways that regulate inflammatory responses. Thus, Berenil might be a viable novel agent for use in the treatment of various inflammatory conditions. In addition, the therapeutic aspects of trypanosomiasis need alternative approaches as

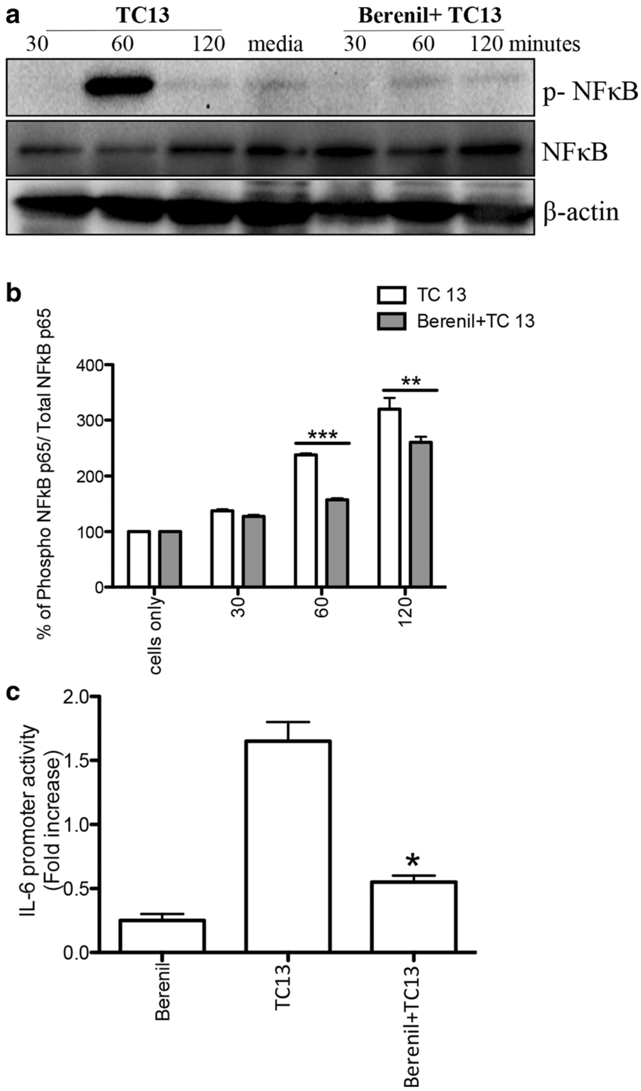

Fig. 5 Berenil downregulates TC13-induced phosphorylation of NFκB p65 subunit and IL-6 promoter activity in macrophages. BMDMs were treated with Berenil (10 μg/ml) overnight and then stimulated with TC13 lysate. At indicated times, the cells were lysed and the lysates were assessed by western blot (A) or ELISA (B) for phosphorylation of NFκB p65 subunit. The same blot was stripped and reprobed with antibody against total NFκB and β-actin and used as loading control (A). ANA cells were transiently transfected with IL-6 promoter construct containing luciferase gene and then pretreated with Berenil (10 μg/ml) for 12 h. Thereafter, the cells were stimulated with TC13 lysate for another 12 h and the luciferase reporter activity was measured (C). The data presented are representative of 3 (A and B) or 2 (C) independent experiments with similar results. $^{**}p < 0.01$; $^{***}p < 0.001$

there is no vaccine and no new drugs are being developed. A complete understanding of the mechanism of action of Berenil, particularly the mechanisms through which it blocks the *T. congolense*-induced cytokine production could help increase the efficacy of the drug and reduce unwanted side effect. Our studies show that Berenil treatment significantly downregulated *T. congolense*-induced phosphorylation of MAPK and STAT proteins and NFκB activity in macrophages both in vitro and in vivo. Collectively, they reveal a mechanistic insight through which Berenil downregulates *T. congolense*-induced cytokine production in macrophages. Given that overproduction of proinflammatory cytokines has been linked to acute death of *T. congolense*-infected animals, these findings suggest that targeting these pathways may provide rational approach to managing disease severity and death associated with this infection.

Compliance with ethical standards

The University of Manitoba Animal Care Committee approved all studies in accordance with the regulation of the Canadian Council on Animal Care.

Conflict of interest The authors declare that they have no conflict of interest.

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