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Synthetic oleanane triterpenoids enhance blood brain barrier integrity and improve survival in experimental cerebral malaria

Valerie M. Crowley^{1†}, Kodjo Ayi^{1†}, Ziyue Lu¹, Karen T. Liby², Michael Sporn² and Kevin C. Kain^{1,3,4*}

Abstract

Background: Cerebral malaria (CM) is a severe complication of *Plasmodium falciparum* infection associated with high mortality and neurocognitive impairment in survivors. New anti-malarials and host-based adjunctive therapy may improve clinical outcome in CM. Synthetic oleanane triterpenoid (SO) compounds have shown efficacy in the treatment of diseases where inflammation and oxidative stress contribute to pathogenesis.

Methods: A derivative of the SO 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO), CDDO-ethyl amide (CDDO-EA) was investigated for the treatment of severe malaria in a pre-clinical model. CDDO-EA was evaluated in vivo as a monotherapy as well as adjunctive therapy with parenteral artesunate in the *Plasmodium berghei* strain ANKA experimental cerebral malaria (ECM) model.

Results: CDDO-EA alone improved outcome in ECM and, given as adjunctive therapy in combination with artesunate, it significantly improved outcome over artesunate alone ($p = 0.009$). Improved survival was associated with reduced inflammation, enhanced endothelial stability and blood–brain barrier integrity. Survival was improved even when administered late in the disease course after the onset of neurological symptoms.

Conclusions: These results indicate that SO are a new class of immunomodulatory drugs and support further studies investigating this class of agents as potential adjunctive therapy for severe malaria.

Background

Malaria remains the most important parasitic disease in the world, causing an estimated 225 million cases and 438,000 deaths annually [1]. The greatest burden of severe and fatal disease is borne by children mainly in sub-Saharan Africa [1, 2]. Current elimination efforts have considerably reduced the global malaria burden, however these advances may paradoxically increase the risk of severe and fatal disease as the level of clinical immunity in the population wanes due to decreased parasite prevalence [3, 4].

The mechanisms underlying cerebral malaria (CM) are incompletely understood, however an interaction between both parasite virulence factors and host response determinants play critical roles in the pathobiology of CM. Parasitized erythrocytes sequester in the microvasculature of vital organs, including the brain, and lead to host innate immune responses, including the release of pro-inflammatory cytokines and angiogenic factors, culminating in a dysregulated inflammatory response, endothelial dysfunction and loss of blood brain barrier (BBB) integrity [5–8]. C57BL/6 mice infected with *Plasmodium berghei* strain ANKA serves as an experimental model of cerebral malaria (ECM), where mice develop severe vasculopathy and neurological manifestations [5, 9–13]. While some features differ between ECM and human CM, those involving microvascular leakage

*Correspondence: kevin.kain@uhn.ca

[†]Valerie M. Crowley and Kodjo Ayi contributed equally to the work
¹ S. A. Rotman Laboratories, Sandra Rotman Centre for Global Health, University Health Network-Toronto General Hospital, Toronto, Canada
Full list of author information is available at the end of the article

and BBB dysfunction are especially comparable in mice and humans during ECM and human CM, respectively [14].

Parenteral artesunate is the current first-line therapy for CM; however, the fatality rate of CM remains high at 18–30%, with up to one-third of survivors developing long-term neurocognitive impairments [15–19]. Furthermore, the current efficacy of parenteral artesunate is threatened by the emergence and spread of artemisinin resistance in Southeast Asia [20]. New classes of anti-malarial drugs and new strategies for adjunctive therapy are urgently needed to improve survival and decrease the burden of neurological injury in survivors.

Triterpenoids are a structurally diverse class of organic molecules naturally synthesized by many plants by the cyclization of squalene [21]. Some naturally occurring triterpenoids, such as ursolic and oleanolic acid, possess weak antitumour and anti-inflammatory properties [22, 23]. To improve their potency as pharmacological agents, synthetic oleanane triterpenoids (SO) have been synthesized and represent a promising class of multifunctional drugs that modulate the activity of several signalling networks [24].

SO are potent activators of host immunomodulatory pathways, including nuclear factor-erythroid2-related factor 2 (Nrf2) both in vitro and in vivo [25]. Under homeostatic conditions Nrf2 is bound by its endogenous inhibitor, Kelch-like ECH-associated protein 1 (Keap1) in the cytoplasm, an interaction that targets the complex for ubiquitination and proteasomal degradation. Under conditions of oxidative stress the Nrf2-Keap1 complex dissociates, allowing Nrf2 to translocate into the nucleus, bind to antioxidant response element (ARE) sequences and induce the transcription of anti-oxidant and anti-inflammatory factors that function to reduce toxicity. SO activate the Nrf2/ARE pathway by directly binding to Keap1, thereby activating Nrf2 [26]. Genes containing ARE sequences include haem oxygenase (HO-1), catalase, superoxide dismutase, and nuclear transcription factors, such as peroxisome proliferator-activating receptor (PPAR γ), highlighting the pleiotropic actions of SO [27–29].

Based on in vitro observations and their ability to target numerous pathways, SO have the potential to be broadly applied to chronic and infectious diseases where host inflammation and oxidative stress contribute to pathogenesis. The methyl ester of a potent SO, 2-cyano-3,12-dioxooleana-1,9 (11)-dien-28-oic acid (CDDO-Me) and related triterpenoids are currently in clinical trials for pulmonary arterial hypertension (clinicaltrials.gov NCT02036970, NCT02657356), Alport Syndrome (NCT03019185) and chronic kidney disease (NCT01053936), and various forms of cancer [30,

31]. Other derivatives, including CDDO-ethyl amide (CDDO-EA) have been formulated for improved bioavailability and to better cross the BBB [32, 33]. While the pathogenesis of malaria is complex, it is characterized by marked inflammation, oxidative stress and their impact on microvascular function [34].

It was hypothesized that SO would improve outcome alone or as combination therapy in the treatment of severe malaria. To test this hypothesis, the effect of CDDO-EA was examined in vivo in a murine model of ECM. This study tested for the effect of CDDO-EA on host response including inflammation, endothelial dysfunction and BBB leak.

Methods

Mice

The University Health Network Animal Use Committee approved all experiments in accordance with institutional guidelines (AUP 1920). Male and female 7–9 weeks old C57BL/6 mice were purchased from Jackson Laboratories (West Grove, PA, USA) and housed under pathogen-free conditions with a 12-h light cycle.

Infection with *Plasmodium berghei*

Plasmodium berghei ANKA (PbA) parasites (MR4; American Type Culture Collection, Manassas, VA, USA) were cultivated by passage through C57BL/6 mice as described [35]. Experimental infections were initiated by intraperitoneal (ip) injection of 1×10^6 parasitized erythrocytes (day 0). Parasite burdens were monitored daily starting on day 3 post-infection for up to 14 days by Giemsa-stained blood smears. Mice were inoculated on the same day, within 30 min and inoculation alternated between control and experimental groups (e.g. control 1, experimental 1, control 2, experimental 2). Mice were assessed for signs of ECM using a modified rapid murine coma and behaviour scale (RMCBS) [36] and were euthanized when moribund according to institutional guidelines [37].

Reagents and drug treatments

For in vivo experiments, CDDO-EA [25, 32] was dissolved in vehicle containing DMSO, Cremophor EL (Sigma-Aldrich) and PBS at a 1:1:8 ratio. Adjunctive therapy with CDDO-EA preparation (200 μ mol/kg) and artesunate (5 mg/kg; Sigma-Aldrich) were co-administered. All treatments were a single dose, administered by an ip injection, in a total volume of 0.2 mL. Treatment was initiated on day 4 or 6 post-infection. Treatment on day 4 corresponds to established infection prior to the development of severe disease, whereas treatment on day 6 corresponds to the onset of neurological manifestations (after BBB leak and the onset

of neurological symptoms) [37] defined as a RMBCS of $\leq 50\%$. On day 6, clinical manifestations of ECM include ruffled hair, hunched body position, inactivity, poor balance, reduced limb strength, reduced touch escape and ataxic gait [36].

Analysis of blood brain barrier integrity

On day 6 post-infection, mice were injected ip with 300 μL of 2% Evans blue. After 2 h, mice were euthanized using isoflurane, and perfused with 20 ml of PBS. Brains were collected, photographed and placed in formamide for 48 h to extract Evans blue dye. Evans blue dye was quantified using a spectrophotometer at 605 nm and compared to a standard curve.

Measurement of transcript levels

Transcripts were measured by quantitative real-time PCR (qRT-PCR). Total RNA was isolated from snap frozen brain tissue after homogenization in TRIzol (1 mL/100 mg tissue; Invitrogen, Burlington, ON, USA) according to manufacturer's instructions. Extracted RNA (1 $\mu\text{g}/\text{sample}$) was treated with DNase I (Fermentas, Burlington, ON, USA), and reverse transcribed to cDNA (BioRad, Mississauga, ON, USA). cDNA was amplified in triplicate with SYBR Green master mix (Roche, Laval, QC, Canada) in the presence of 1 μM of forward and reverse primers in a Light Cycler 480 (Roche, Laval, QC, Canada). Transcript expression levels were calculated compared to a standard curve of mouse genomic DNA included on each plate and normalized to average GAPDH expression levels. The primer used are as follows: for Nrf-2 (mouse), 5'-TCTCCTCGCTGGAAAAAGAA-3' and 5'AATGTGCTGGCTGTGCTTTA-3' and for HO-1 (mouse), 5'-AACAAGCAGAACCAGTCTATGC-3' and 5'-AGG-TAGCGGGTATATGCGTGGGCC-3', GAPDH (mouse) 5'-TCAACAGCAACTCCCCTCTTCCA-3' and 5'-TTGTCATTGAGAGCAATGCCAGCC-3', GAPDH (human) 5'-GCCTCAAGATCATCAGCAATGC-3' and 5'-CCTTCCACGATAACAAAGTTGTCAT-3', Nrf-2 (human) 5'-CCTCAACTATAGCGATGCTGAATCT-3' and 5'-AGGAGTTGGGCATGAGTGAGTAG-3', HO-1 (human) 5'-GCAGAGAATGCTGAGTTCATG-3' and 5'-CACATCTATGTGGCCCTGGAGGAGG-3'.

Measurement of cytokines and markers of endothelial activation

Cytokines and markers of endothelial activation were measured using an enzyme-linked immunosorbent assay (ELISA) on day 6 post-infection on mice that were drug-treated on day 4 post-infection. Blood was collected in heparin-coated tubes by cardiac puncture and assayed for interleukin-10 (IL-10), tumour necrosis factor (TNF),

interferon- γ (IFN- γ), and angiopoietin-1 (Ang-1) using commercially available standard ELISA kits according to the manufacturer's instructions (Duosets, R&D Systems, Minneapolis, MN, USA). Ang-2 was measured using a Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA).

Isolation of peripheral blood mononuclear cells

Human peripheral blood mononuclear cells (PBMCs), 5×10^5 cells/well were treated with 1 μM dihydro-artemisinin (DHA) dissolved in DMSO. After incubation for 24 h at 37 $^{\circ}\text{C}$, supernatants were removed and cells were resuspended directly in TRIzol for Nrf2 and HO-1 mRNA quantification by qRT-PCR. Fresh PBMCs were obtained from healthy blood donors. All blood donors were provided with written informed consent by a protocol approved by the University of Toronto, Protocol Reference #21081.

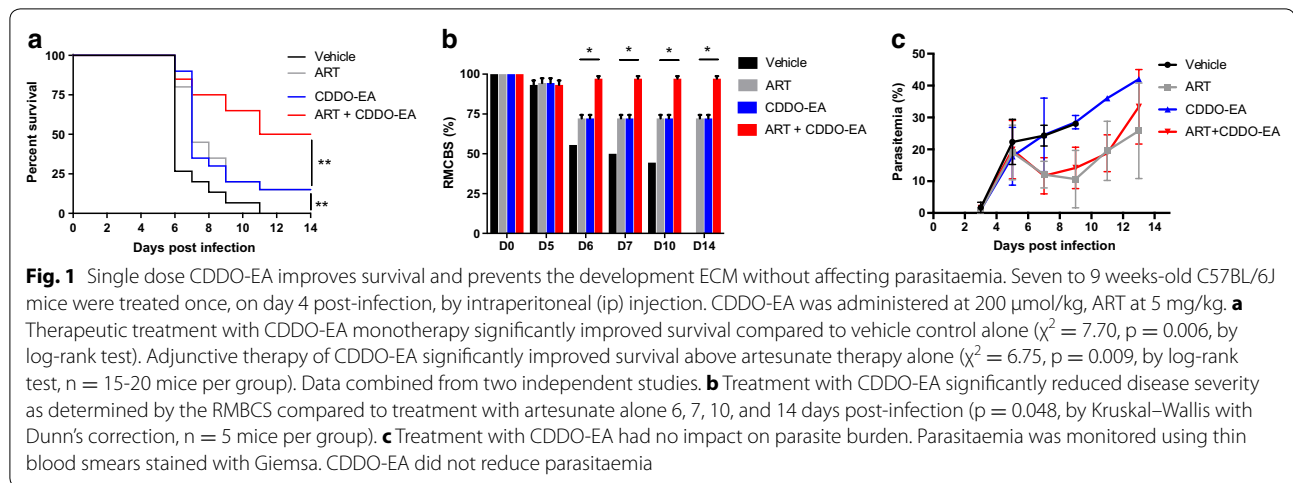
Statistical analysis

A log-rank test was performed on the Kaplan–Meier survival curves. The Kruskal–Wallis H test was performed on RMBCS, mRNA levels, ELISAs and Evans blue data and multiple comparisons were corrected using Dunn's correction. A *t* test was carried out on the transcript levels from PBMCs. Tests were performed with Prism version 7.0b (Macintosh version GraphPad Software Inc, La Jolla, CA, USA).

Results

Mono- and adjunctive CDDO-EA therapy improves outcome in experimental cerebral malaria

To determine whether therapeutic administration of CDDO-EA would improve outcome in C57BL/6J mice susceptible to ECM, PbA-infected mice were either treated with a single dose of CDDO-EA alone or in combination with artesunate. CDDO-EA monotherapy on day 4 post-infection significantly improved survival compared to vehicle control ($p = 0.006$; Fig. 1a). Survival with CDDO-EA monotherapy was equivalent to that of mice treated with artesunate alone. When single dose CDDO-EA was administered adjunctively with artesunate on day 4 post-infection, survival was significantly improved over that of artesunate treatment alone ($p = 0.009$; Fig. 1a). Mice treated with CDDO-EA in combination with artesunate did not develop neurological manifestations of ECM as assessed by RMBCS (Fig. 1a). The improved survival and reduced disease severity with CDDO-EA intervention was not associated with changes in parasite burden as determined by peripheral parasitaemia (Fig. 1c).



CDDO-EA monotherapy increases *Nrf2* and *HO-1* levels but is counteracted by artemisinin

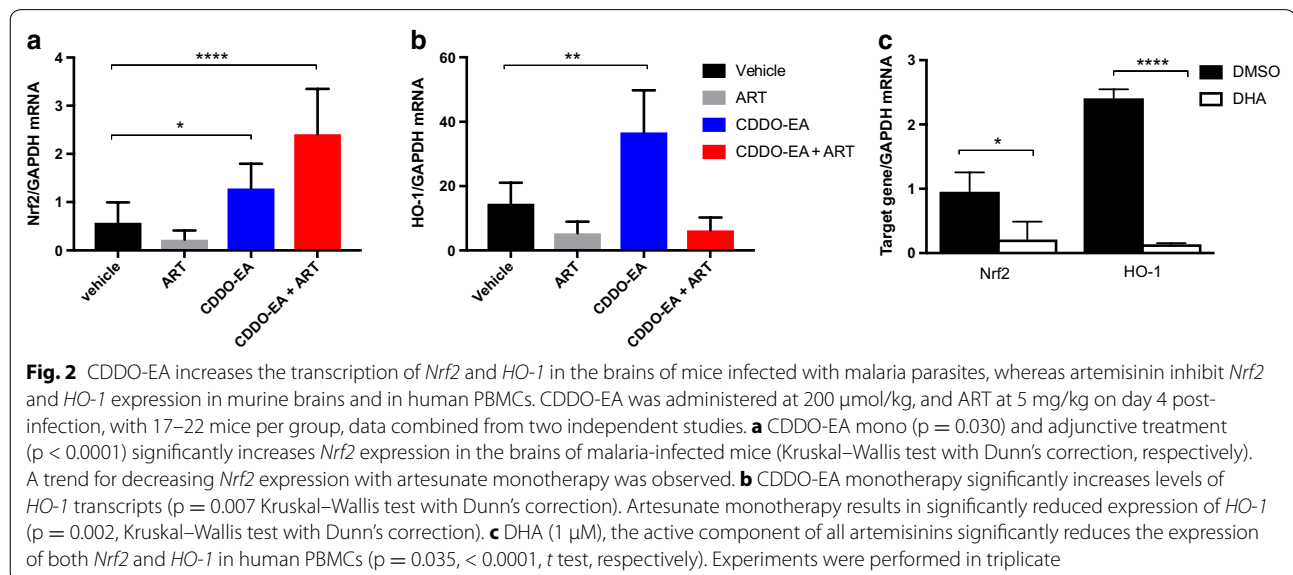
SO are potent activators of the transcriptional regulator *Nrf2* [25] and *HO-1*, a transcriptional target of *Nrf2*, has been implicated in the pathogenesis of ECM [38]. Therefore, the levels of *Nrf2* and *HO-1* transcripts were assessed in the brains of PbA-infected mice on day 6 post-infection.

Increased levels of *Nrf2* and *HO-1* transcripts were observed in mice treated with CDDO-EA monotherapy ($p = 0.030$) and CDDO-EA in combination with artesunate ($p \leq 0.0001$), whereas artesunate alone decreased the levels of *Nrf2* and *HO-1* compared to vehicle alone (Fig. 2a, b).

To determine whether the inhibitory effects of artesunate on *Nrf2* and *HO-1* expression were also observed in human cells, PBMCs were incubated with and without DHA, the active metabolite of artemisinins. PBMCs incubated with DHA had significantly reduced expression of *Nrf2* and *HO-1* compared to control-treated cells ($p = 0.035$, < 0.0001 , respectively, Fig. 2c).

Mono- and adjunctive CDDO-EA therapy reduces cytokine levels and improves endothelial stability

Immune and endothelial dysfunction have been implicated in the pathogenesis of CM in humans and in ECM [8]. Dysregulation of plasma angiopoietin levels is associated with disease severity and predicts clinical outcome



in human malaria [37, 39–41]. Increased survival of mice treated adjunctively with CDDO-EA was associated with decreased levels of plasma IL-10 ($p = 0.003$), TNF and IFN- γ ($p = 0.018$, Fig. 3). CDDO-EA treatment alone and in combination with artesunate resulted in enhanced endothelial stability [37] as determined by increased Ang-1 (adjunctive, $p = 0.014$), decreased Ang-2 levels ($p = 0.030$ and 0.049 , monotherapy and adjunctive therapy respectively) and lower Ang-2/Ang-1 ratio compared to vehicle alone (adjunctive, $p = 0.012$, Fig. 4).

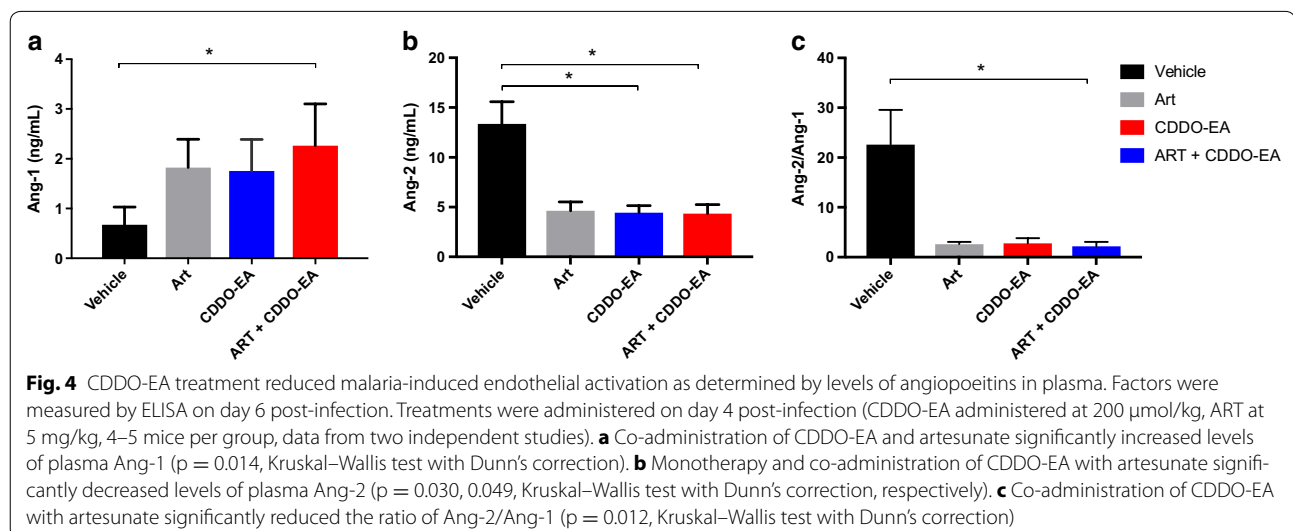
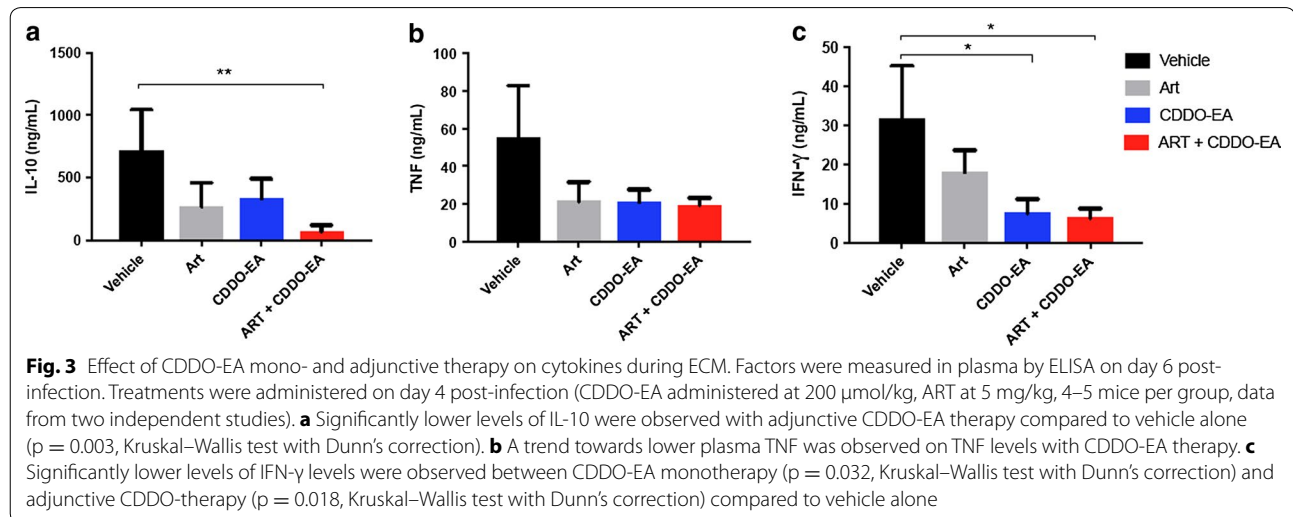
Mono- and adjunctive CDDO-EA therapy enhances BBB integrity

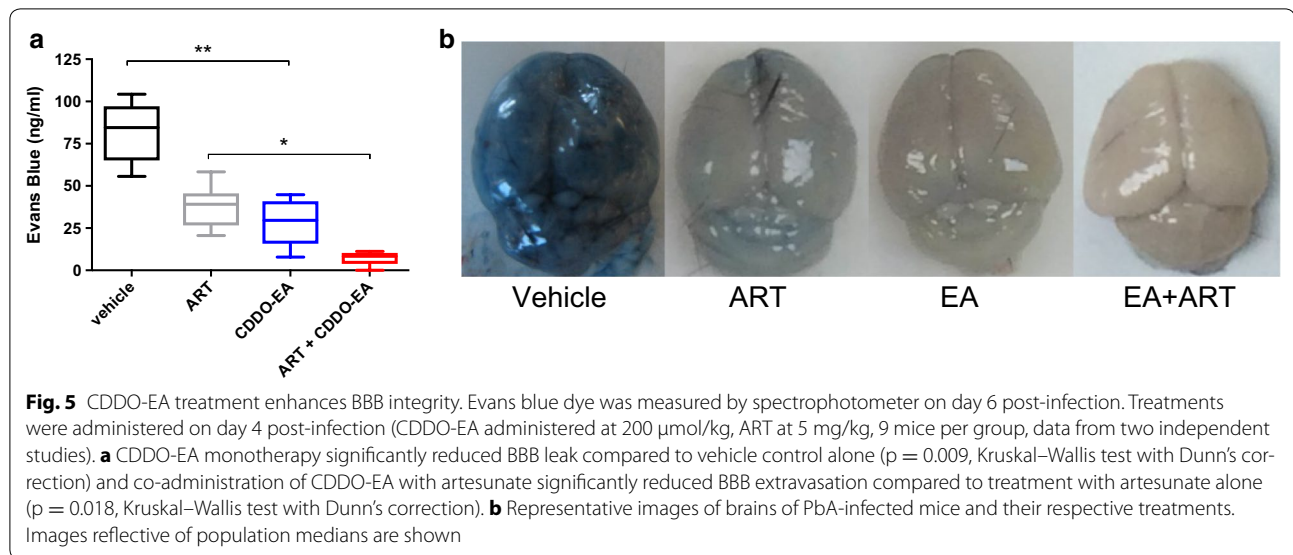
BBB leak is a hallmark of both ECM and paediatric CM [14, 42–45]. The improved survival of PbA-infected mice treated with CDDO-EA alone and in combination

with artesunate was associated with preservation of the BBB as assessed by quantification of Evans blue extravasation into brain parenchyma (Fig. 5a, b). Compared to untreated mice, CDDO-EA alone significantly reduced cerebrovascular leak compared to vehicle control ($p = 0.009$). This reduction in leak was comparable to that obtained with artesunate treatment. Adjunctive CDDO-EA enhanced BBB integrity beyond that obtained with artesunate treatment alone ($p = 0.018$).

Adjunctive CDDO-EA treatment improves survival compared to artesunate alone when administered to mice with neurological impairment

To better mimic a clinically relevant scenario when children present with neurological impairment, mice were treated with CDDO-EA alone and adjunctively at the





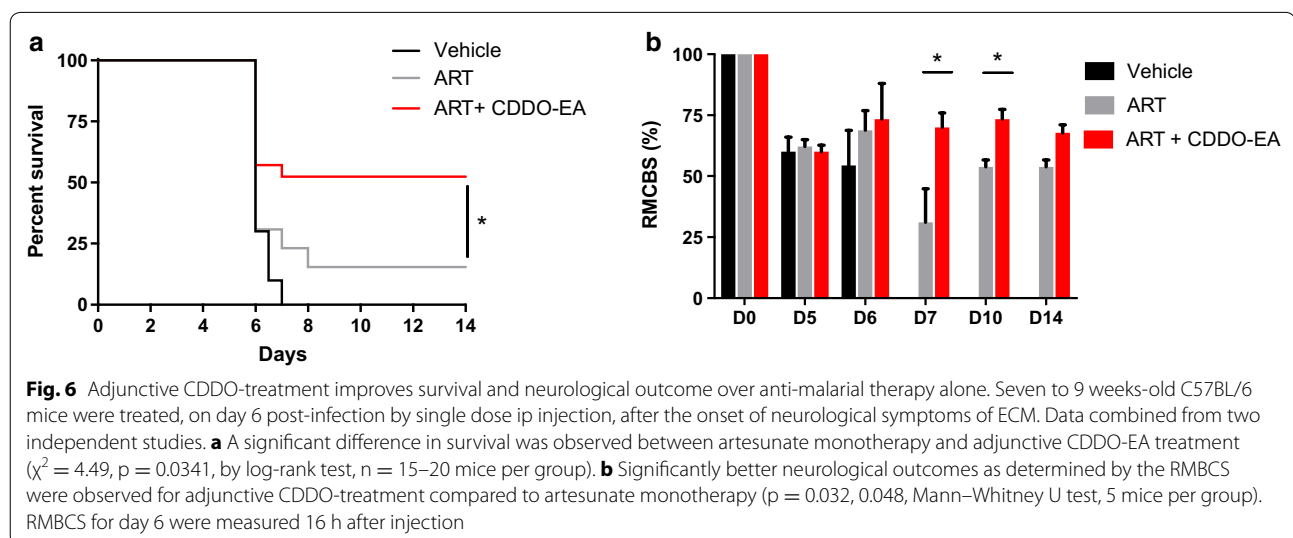
onset of neurological symptoms. Survival was significantly improved with adjunctive CDDO-EA treatment compared to artesunate alone ($p = 0.0341$, Fig. 6a). Mice receiving adjunctive CDDO-EA also displayed significantly reduced disease severity at days 7 and 10 post-infection as determined by the RMCBS ($p = 0.032$ and 0.048 respectively, Fig. 6b).

Discussion

SO are a class of drugs that has shown promise in the treatment of chronic, non-communicable diseases where pathogenesis is linked to inflammation and oxidative stress [25]. A pre-clinical model of severe malaria, shows

the potentially utility of CDDO-EA as an adjunctive therapy for a life-threatening infection.

SO regulate Nrf2-activity by releasing its interaction with its inhibitor Keap-1, allowing for the translocation of Nrf2 to the nucleus and for the transcription of Nrf2 target genes [24, 46, 47]. An observed increase in Nrf2 transcription in the brains of PbA-infected mice upon CDDO-EA treatment, likely reflects autoregulation of Nrf2 that has been observed in vitro and in kidneys of mice treated with CDDO-Im [48, 49]. CDDO-EA was administered only once and this Nrf2 positive feedback loop may be beneficial in prolonging the protective effects of a single dose of CDDO-EA. Of particular interest to ECM is the Nrf2-target gene *HO-1*, which has been



shown to modify the pathogenesis of ECM and improve outcome and CDDO-EA treatment alone increased levels of *HO-1* [38]. The data suggests that adjunctive CDDO-EA therapy improves survival through a Nrf2-dependent, HO-1 independent mechanism. However importantly, artesunate and DHA, first-line therapy for human falciparum malaria, inhibit *Nrf2* and *HO-1* expression in both human PBMCs and in malaria-infected mice. Of note, co-treatment with CDDO-EA overcomes the inhibition of *Nrf2* by artemisinin but not that of *HO-1* in ECM. Whether the decrease of *Nrf2* and *HO-1* expression associated with artemisinin treatment has implications for cellular recovery, detoxification and neurological outcomes in severe and CM in humans remains to be determined but theoretically inhibiting these pathways could have a negative impact on malaria outcomes [38, 49, 51, 70, 71]. Clinical trial evidence indicates that neurocognitive deficits in children treated with parenteral artesunate for CM remain high at up to 35% of survivors [50] and it will be of interest to evaluate whether CDDO-EA treatment can mitigate this high level of neurological impairment in pre-clinical or ultimately clinical studies [51].

The immuno-regulatory cytokine IL-10 plays a protective role in the development of ECM [52–54]. CDDO-EA therapy has been shown to increase levels of IL-10 in an experimental murine model of chronic liver injury [55]. Although CDDO-EA improves the survival of ECM-susceptible mice, it appears to do so through an IL-10-independent mechanism, as treatment reduced IL-10 levels

in Pba-infected mice. Although high levels of IL-10 may play a beneficial role in human malaria by inhibiting parasite-induced pro-inflammatory responses that contribute to disease severity [56], studies have [57, 58] reported that high levels may also impair malaria clearance [59]. The development of severe malaria is associated with a dysregulated host immune response to infection characterized by increased circulating levels of TNF and IFN- γ in both humans [60–63] and murine models of ECM [54]. To determine if CDDO-EA was able to modify the host inflammatory response, the levels of these pro-inflammatory cytokines were assessed in ECM. CDDO-EA treatment alone led to significantly reduced levels of both IFN- γ and TNF, in agreement with previous observations in a murine model of cirrhosis [55]. It is possible that a single time point fails to capture crucial dynamic differences of these mediators between experimental groups, and most mice died on day 7 post-infection rather than day 6 when the measurements were made. Of note anti-inflammatory adjunctive therapies (e.g. dexamethasone, anti-TNF strategies) have been shown to improve outcome of human CM; with some reported to result in prolonged coma and worse clinical outcomes [64–68].

The angiotensin-Tie2 axis is a critical regulator of endothelial integrity and microvascular leak [69]. Ang-1 binds to and induces the phosphorylation of the Tie2 receptor kinase on endothelial cells. This binding contributes to vascular quiescence and stability. In response to inflammation, Ang-2 is released by endothelial cells,

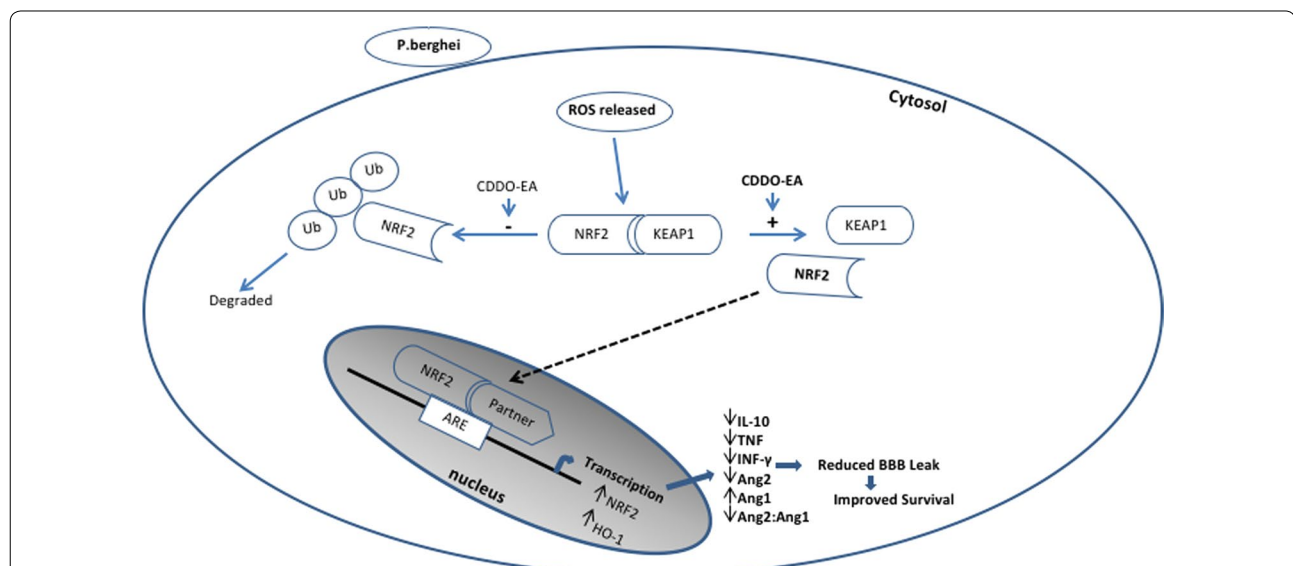


Fig. 7 Summary of the protective effects of CDDO-EA in ECM. Infection by *P. berghei* leads to oxidative stress and inflammation. CDDO-EA disrupts the interaction of Nrf2 and Keap1, allowing for the translocation of Nrf2 to the nucleus. With its binding partners, Nrf2 initiates the transcription of genes containing ARE elements in their promoters. In ECM, CDDO-EA treatment reduced levels of IL-10, TNF, INF- γ , Ang-2 while increasing levels of Ang-1, resulting in reduced BBB leak and improved survival

and competitively binds to Tie2, where it promotes vascular permeability and instability [43, 44]. Absolute levels and the ratio between Ang-1 and Ang-2 are associated with disease severity and fatality in CM [39–41, 70]. Ang-1 has recently been shown to play a mechanistic role in the pathogenesis of cerebral malaria and adjunctive treatment with Ang-1 can improve BBB integrity and survival in experimental CM even in the face of high circulating levels of inflammatory cytokines [37]. Adjunctive treatment with CDDO-EA significantly increased levels of Ang-1, and decreased both the levels Ang-2 and the ratio of Ang-2/Ang-1 compared to vehicle control alone. These findings are consistent with the hypothesis that CDDO-EA may improve survival of PbA-infected mice at least in part by via Ang-1 enhanced endothelial and BBB integrity (Fig. 7).

Reduced BBB leak was observed in mice treated with CDDO-EA. Intravital microscopy in PbA-infected mice has shown that neurological symptoms of ECM are associated with breakdown of the neuro-immunological BBB [45] and disruption of microvascular permeability in humans has been associated with fatal CM [8, 42, 71]. CDDO-EA was initially synthesized to increase BBB permeability and has been shown to cross the BBB in mice [33]. Mice with established infection that were treated with CDDO-EA before the onset of neurological symptoms showed improved survival and did not develop neurological signs of ECM. Adjunctive treatment with CDDO-EA before the onset of neurological symptoms significantly reduced BBB leak above that of artesunate treatment alone and prevented mice from developing neurological signs of ECM, as assessed by the RMBCS.

To mimic a more clinically relevant scenario where patients often seek treatment late in the course of disease, mice were treated after the onset of neurological symptoms with adjunctive CDDO-EA co-administered with artesunate. We did not assess CDDO-EA as a monotherapy at this stage as parenteral artesunate is the current first line treatment for severe malaria [72, 73]. This treatment increased survival and improved the neurological outcome of mice already displaying neurological signs, suggesting that adjunctive CDDO-EA may not only prevent BBB leak but also restore its function and mitigate associated neurological injury. Rapid reversibility of cerebral swelling has also been observed in adults and children after anti-malarial treatment and highlights the need for new malarial treatments that can rapidly prevent and reverse neuronal cytotoxicity [71]. These findings are in agreement with neuroprotective effects observed with triterpenoids used in pre-clinical models of Huntington's disease and induced neurotoxicity [74, 75].

CDDO-EA did not reduce peripheral parasitaemia levels and all mice that survived ECM went on to develop hyperparasitaemia and anaemia. This indicates that CDDO-EA does not have an anti-parasitic effect on PbA in vivo and its mechanism of action is via modifying host response to infection. These observations are consistent with a growing literature in both pre-clinical models and human infections that host response is a major determinant of outcome and that interventions targeting host response may improve survival and reduce disease severity over antimicrobial therapy alone [76, 37, 40].

CDDO is a partial agonist of PPAR γ and this activity may also contribute to improved survival in ECM. CDDO acts by preventing the binding of a nuclear receptor co-repressor (NCoR) and by recruiting CCAAT/enhancer-binding protein (CBP/p300), a transcriptional co-activator to PPAR γ [29]. Rosiglitazone, a PPAR γ agonist, has been investigated as an adjunctive therapy in ECM where it improves survival, reduces inflammation, prevents vascular leak, and prevents memory and learning deficits in mice with ECM [49]. A randomized, double blind, placebo-controlled trial of rosiglitazone in young adults with uncomplicated malaria resulted in increased parasite clearance times and increased circulating levels of endothelial stabilizing and neuroprotective factors, including Ang-1 and brain-derived neurotrophic factor (BDNF), respectively [51]. A safety and tolerability of rosiglitazone in children with uncomplicated malaria has been completed [72] and is currently being extended to a Phase IIb clinical trial of paediatric severe malaria in Mozambique.

CDDO and its derivatives are currently being investigated in murine models of neurodegenerative disorders such as Amyotrophic lateral sclerosis (ALS) and Huntington's disease where improvements to memory and motor impairments have been observed [33, 75]. Whether the improved survival in ECM observed with adjunctive CDDO-EA will also be accompanied by reduced cognitive and motor impairments in surviving mice is unknown. It will be important to explore the potential neuroprotective effects of CDDO-EA in future studies. In particular, since CDDO derivatives have been reported to be neuroprotective in several preclinical models, the unique attribute of CDDO-EA to overcome the artemisinin inhibition of Nrf2 may be of utility as an adjunctive therapy for CM.

Conclusion

In summary, single dose treatment with CDDO-EA improved the survival of ECM-susceptible mice when administered before the development of ECM. This improved survival was associated with increased cerebral expression of Nrf2, reduced levels of TNF and

IFN- γ , improved vascular integrity and reduced BBB leak. Importantly CDDO-EA improved survival and disease progression when administered after the onset of neurological symptoms. These results support investigation of CDDO-EA and other SO as potential adjunctive therapy for severe malaria.

Abbreviations

ALS: amyotrophic lateral sclerosis; Ang: angiotensin; ARE: antioxidant responsive element; ART: artesunate; BBB: blood brain barrier; CDDO: 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid; CM: cerebral malaria; DHA: dihydroartemisinin; EA: ethyl amide; ECM: experimental cerebral malaria; ELISA: enzyme-linked immunosorbent assay; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; HO-1: haem oxygenase-1; IFN- γ : interferon-gamma; IL-10: interleukin-10; Nrf2: nuclear factor (erythroid-derived 2)-like 2; Pba: *Plasmodium berghei* ANKA; PBMC: peripheral blood mononuclear cell; PPAR γ : peroxisome proliferator-activated receptor gamma; RMCBS: rapid murine coma and behaviour score; SO: synthetic oleanane triterpenoids; TNF: tumour necrosis factor.

Authors' contributions

KL, MS and KCK conceived of the study and VMC and KA contributed to study design. VMC, KA and ZL acquired the data. VMC, KA and KCK analysed and interpreted the data. VMC, KA and KCK drafted the manuscript. VMC, KA, KL, MS, and KCK critically revised the manuscript. All authors read and approved the final manuscript.

Author details

¹ S. A. Rotman Laboratories, Sandra Rotman Centre for Global Health, University Health Network-Toronto General Hospital, Toronto, Canada. ² Department of Pharmacology, Dartmouth Medical School, Hanover, NH, USA. ³ Department of Medicine, University of Toronto, Toronto, ON, Canada. ⁴ Tropical Diseases Unit, Division of Infectious Diseases, Department of Medicine, UHN-Toronto General Hospital, Toronto, ON, Canada.

Competing interests

MS and KL have patent interest in synthetic triterpenoids. The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Consent for publication

Not applicable.

Ethics approval

The University Health Network Animal Use Committee approved all experiments in accordance with institutional guidelines (AUP 1920).

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