

Ceylon cinnamon and its major compound Cinnamaldehyde can limit overshooting inflammatory signaling and angiogenesis *in vitro*: implications for COVID-19 treatment

Kurt Lucas^{*1}, Maximilian Ackermann^{2,3}, Anna Lena Leifke¹, William W. Li⁴,
Ulrich Pöschl¹, Janine Fröhlich-Nowoisky¹

¹Max Planck Institute for Chemistry, Multiphase Chemistry Department, Mainz, Germany

²Institute of Pathology and Molecular Pathology, Helios University Clinic Wuppertal,
University of Witten/Herdecke, Wuppertal, Germany

³Institute of Functional and Clinical Anatomy, University Medical Center of the Johannes
Gutenberg-University Mainz, Mainz, Germany

⁴ The Angiogenesis Foundation, Cambridge, Massachusetts, United States of America

* Corresponding author

Email k.lucas@mpic.de (KL)

17

18 **Keywords**

19 Ceylon cinnamon extract (CCE)

20 Cinnamaldehyde (CA)

21 Coronavirus disease 2019 (COVID-19)

22 Dexamethasone

23 Toll-like receptor 4 (TLR4)

24

25 **Abbreviation list**

26 CCE: Ceylon cinnamon extract

27 CE: cinnamaldehyde

28 CRP: C-reactive protein

29 DAMP: damage-associated molecular pattern

30 Dex: dexamethasone

31 HMGB1: high mobility group box 1 protein

32 HSP60: heat shock protein 60

33 HUVEC: human umbilical vein endothelial cells

34 IFN- γ : interferon gamma

35 IL-1 β : interleukin 1 beta

36 RAGE: receptor for advanced glycation endproducts

37 ROS/RNS: reactive oxygen and nitrogen species

38 TLR4: toll-like receptor 4

39 TNF: tumor necrosis factor

40

Abstract

Overshooting immune reactions can occur during inflammatory responses that accompany severe infections, such as COVID-19. Cytokines, damage-associated molecular patterns (DAMPs), and reactive oxygen and nitrogen species can generate positive feedback loops of inflammation, leading to long-term complications such as vascular endothelialitis, thrombosis, endothelial dysfunction, neurological impairments, and chronic fatigue. Dexamethasone can limit inflammation by inhibiting the activation of pro-inflammatory transcription factors. High dose dexamethasone, however, has undesirable side effects. Here, we show that Ceylon cinnamon and its major compound cinnamaldehyde can mitigate inflammatory signaling *in vitro*. Cinnamaldehyde interferes with the dimerization of toll-like receptor 4 (TLR4), which can be activated by DAMPs like HSP60 and HMGB1. Our results suggest that supplementary treatment with Ceylon cinnamon may allow administration of lower doses of dexamethasone to avoid high dose steroid side effects. Moreover, preliminary results indicate that Ceylon cinnamon modulates angiogenesis, which is a reactive phenomenon in COVID-19.

Introduction

Coronavirus disease 2019 (COVID-19) causes an acute inflammatory response in organs with frequent long-term effects. Ackermann et al. 2020 described massive pulmonary vascular endothelialitis, thrombosis, and angiogenesis in COVID-19 patients (1). Long-term complications include neurological disturbances, kidney and myocardial disorders, as well as chronic fatigue (2-5). COVID-19 infection is also characterized by excessive immune reactions, which are frequently referred to as the 'cytokine storm' (6, 7). Various cytokines are involved in this overshooting immune reaction, including TNF, IFN- γ , IL-1 β , IL-2, IL-4, IL-6, IL-8, and CRP (8-10). The transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B, p50/p65) is also heavily involved in inflammatory processes (Fig.

(mod-DAMPs) with enhanced TLR4-stimulating potential, additionally fueling inflammation. Dexamethasone inhibits activation of NF- κ B, whereas cinnamaldehyde, the major active compound of ethanolic Ceylon cinnamon extract, inhibits activation of TLR4, thus disrupting the feedback loops.

One of the clinical goals for the treatment of COVID-19 is to mitigate an excessive inflammatory response to SARS-CoV-2 infection. Dexamethasone, a corticosteroid which acts on NF- κ B (22, 23) is an effective therapeutic intervention for moderate to severe disease (4, 24), but it can cause unwanted side effects in high doses. TLR4 could be another potential target for treatment of COVID-19 since it plays an important role in the DAMP-driven positive feedback loops (Fig. 1). Cinnamaldehyde (CA), the major active compound of Ceylon cinnamon (*Cinnamomum verum*) extract (CCE) (25-27) can inhibit the activation of TLR4 by preventing receptor dimerization (28). As shown in an animal model, the concurrent intake of Cinnamon can also mitigate the side effects of dexamethasone (29). We hypothesized that Ceylon cinnamon or cinnamaldehyde might be well suited as a supplementary treatment for COVID-19 to lower the risk of the 'cytokine storm' and allow administration of reduced dosages of dexamethasone to mitigate steroid-induced side-effects (30).

In this study, we compared CCE and CA with dexamethasone in the suppression of TLR4-dependent cytokine mRNA production and IL-8 protein release *in vitro*. Further, we present preliminary data showing that CCE inhibits tube formation, which serves as a cellular model for angiogenesis.

We do not recommend the use of Cassia cinnamon or its extracts, due to its possibly high content of liver-toxic coumarin (31).

Materials and Methods

Ceylon cinnamon extract and cinnamaldehyde

Ceylon cinnamon extract (CCE) was prepared by extraction from Ceylon cinnamon powder (*Cinnamomi ceylanici cortex*, Caesar & Loretz GmbH, Hilden, Germany) with 70% ethanol (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) in a 1:5 ratio and incubation for 10 days at room temperature, protected from light and shaken once daily. After the incubation period, the CCE was filtered through a 0.22-µm pore size PES membrane filter unit (Corning Inc., Corning, USA) under sterile conditions and stored in brown glass bottles at room temperature protected from light. In a previous study, a CCE concentration of 0.3% was determined to be optimal for the treatment of THP-1 cells (26), with higher concentrations resulting in cytotoxic effects; lower concentrations were, on the other hand, less potent in preventing inflammation (26). CCE was diluted 1:10 in Dulbecco's Phosphate Buffered Saline (DPBS -/-) (Thermo Fisher Scientific Inc., Waltham, USA) and cells were treated with final concentrations of 0.2% and 0.3% CCE.

A cinnamaldehyde (CA, Sigma-Aldrich) stock solution of 20 mg/mL was freshly prepared in absolute ethanol (Sigma-Aldrich). For cell culture experiments, we diluted the CA stock solution in DPBS -/- to concentrations of 1 mM and 10 mM.

Dexamethasone

A dexamethasone (Sigma-Aldrich) stock solution (1 mg/mL in 70% ethanol) was freshly prepared for each experiment. THP-1 cells were treated with final dexamethasone concentrations of 3.2 µM and 6.4 µM according to Menacher et al., 2017 (32).

Cultivation and treatment of THP-1 monocytes

Cell culture experiments were performed with human THP-1 acute monocytic leukemia cells (ATCC, LGC Standards GmbH, Wesel, Germany). Cells were grown in Roswell Park

Memorial Institute (RPMI) 1640 medium (Thermo Fisher Scientific) supplemented with 10% heat-inactivated fetal bovine serum (FBS superior; Biochrom, Berlin, Germany), 0.05 mM 2-mercaptoethanol (Sigma-Aldrich) and 100 U/mL penicillin-streptomycin (Thermo Fisher Scientific) at 37 °C in a 5% CO₂ humidified atmosphere.

The THP-1 cells were seeded in 96-well V-bottom cell culture plates (Greiner Bio-One GmbH, Frickenhausen, Germany) at a density of 4x10⁴ cells in 100 µL growth medium per well and allowed to settle for 1 h. In duplicate, cinnamon extract or vehicle control (70% ethanol) was added to the cells in final concentrations of 0.2% and 0.3%, and cells were pre-incubated for 2 h. Cinnamaldehyde was added in concentrations ranging from 10 µM up to 70 µM, also including a vehicle control (100% ethanol), followed by pre-incubation in the same way. After pre-incubation, TLR4 activation was stimulated by adding lipopolysaccharide (LPS-EB from *E. coli* 0111:B4; InvivoGen, Toulouse, France) in a final concentration of 50 ng/mL. Cells were incubated with LPS for 4 h. Supernatants were used to determine IL-8 release using an enzyme-linked immunosorbent assay (ELISA; BD, Heidelberg, Germany). Cell viability was determined following overnight incubation with alamarBlue™ cell viability reagent (Thermo Fisher Scientific), which was added to the cells in a concentration of 10%. After incubation, the fluorescence intensity was measured with a Synergy™ NEO HTS multi-mode microplate reader (Biotek Instruments GmbH, Bad Friedrichshall, Germany) using excitation and measurement wavelengths of 560 nm and 590 nm, respectively. Two independent experiments were performed.

For qPCR analysis, cells were seeded in 6-well cell culture plates at a density of 4x10⁵ cells/mL and treated in triplicate with 0.3% cinnamon extract, 3.2 µM or 6.2 µM dexamethasone, and CA in a range of 20 µM–50 µM plus vehicle control as described above. LPS incubation was carried out for 1 h and 4 h. After incubation, cells were separated from supernatants by centrifugation (500 x g, 5 min), lysed in RLT buffer (RNeasy Mini Kit;

Qiagen, Hilden, Germany) and stored at -80 °C for RNA extraction and real-time quantitative PCR. Two independent experiments were performed on different days.

IL-8 ELISA

Interleukin-8 secretion of treated THP-1 monocytes was quantified by the ELISA OptEIA™ Set for human interleukin-8 (BD) following the manufacturer's protocol with optimized wash buffer composition (50x dilution). The cell supernatants were diluted in a range of 1:2–1:20 and tested in triplicate. The absorbance wavelength of 450 nm and reference wavelength of 570 nm were determined on a Synergy NEO plate reader. Using Gen5 software (Biotek), the reference wavelength values were subtracted from the absorbance wavelength values. Based on measured standard values, a standard curve was constructed. IL-8 cytokine production was calculated according to the standard curve and respective dilution factors. Statistical analysis and corresponding graphs were generated with Prism 9 software (GraphPad Software, San Diego, USA).

RNA extraction and qPCR

Real-time quantitative PCR (qPCR) was performed to quantify mRNA expression of IL1-β, IL-8, and TNF. Extraction of total RNA from cells was performed using the RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. Purified RNA was eluted with 30 µL RNase-free water. Total RNA yield was determined by measuring absorbance at 260 nm, 280 nm, and 320 nm using Take3 Trio Micro-Volume Plates (Biotek) in combination with the Synergy NEO plate reader. All samples were adjusted to an RNA concentration of 50 ng/µL. Single-strand cDNA was synthesized from 500 ng RNA per sample using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific) following the standard protocol. cDNA (2.1 ng) served as template for a qPCR reaction using SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad Laboratories, Hercules, USA) and forward and reverse primers in a final concentration of 333 nM each. Primer sequences were designed with Primer-BLAST software (NCBI) (Table 1). Peptidylprolyl Isomerase A

(PPIA) and TATA-Box Binding Protein (TBP) served as reference genes (33). The following thermal cycling protocol was used: 98 °C for 30 s followed by 40 cycles of 98 °C for 10 s, and 60 °C for 25 s. Data analysis was performed with CFX Manager software 3.1 (Bio-Rad) using the $2^{-\Delta\Delta CT}$ method to calculate gene expression. For qPCR, all samples were tested in technical duplicates and arithmetic mean and standard deviation values were calculated. Prism 9 software (GraphPad Software) was used for statistical analysis.

Cultivation of HUVEC Cells

HUVEC cells (Lonza) were cultivated in endothelial cell medium (EGM-2; Lonza) containing 2% fetal bovine serum (FBS) and vascular endothelial growth factor (VEGF) for rapid proliferation. To analyze tube formation, the cells were first grown (seeding density 2×10^4 cells/mL) in 6-well plates and then transferred to collagen-coated bottom plates without basement membrane extract (CELLCOAT-coated; Biocompare, South San Francisco, CA, USA). The cells were stained with Calcein-AM (#17783, Sigma, Taufkirchen, Germany) and inspected with a fluorescence microscope (Olympus, Hamburg, Germany) using excitation and emission wavelengths of 496 nm and 520 nm.

Endothelial cell tube formation assay

A commercial assay system was used (Thermo Fisher Scientific, Waltham, MA, USA) to determine endothelial cell tube formation; the assay was performed as described in the instructions from the manufacturer and following a published procedure(34). In this system, HUVEC cells (from Lonza, Basel, Switzerland) were cultivated in EGM-Plus Growth Medium (with 5 mM glucose), containing supplements (35) at 37 °C with 5% CO₂. For the experiments, cells at passage <11 were used. The matrix, formed from collagen/basement membrane extract (Geltrex; Thermo Fisher Scientific), was layered into 12 well-plates (Corning/Costar-Sigma, Taufkirchen, Germany). The dishes were overlaid with 1×10^5 cells/well in 400 µL of conditioned medium. Tube formation was checked during the first

10 h using a reflection electron microscope (REM). Electron microscopy was performed with a scanning electron microscope (ESEM) using an ESEM XL-30 apparatus (Philips, Eindhoven; Netherlands).

Table 1 PCR primer sequences. Primer pairs used for qPCR; cytokines: interleukin 1 beta (IL-1 β), interleukin 8 (IL-8), tumor necrosis factor (TNF); reference genes: peptidylprolyl isomerase A (PPIA) and TATA-box binding protein (TBP).

Gene	Accession number	Sequence 5' - 3'	
IL1- β	NM_000576.2	fw	GCCCTAAACAGATGAAGTGCTC
		rv	GAACCAGCATCTTCCTCAG
IL-8	NM_000584.3	fw	AGTCCTTGTTCCACTGTGCCTTGG
		rv	TGCTTCCACATGTCCTCACAACATC
PPIA	NM_021130.4	fw	TCTGCACTGCCAAGACTGAG
		rv	TGGTCTTGCCATTCCTGGAC
TBP	NM_001172085.1	fw	TGAGCCAGAGTTATTTCTGGT
		rv	AATTTCTGCTCTGACTTTAGCACC
TNF	NM_000594.4	fw	GCCCAGGCAGTCAGATCATCTT
		rv	CCTCAGCTTGAGGGTTTGCTACA

Results and Discussion

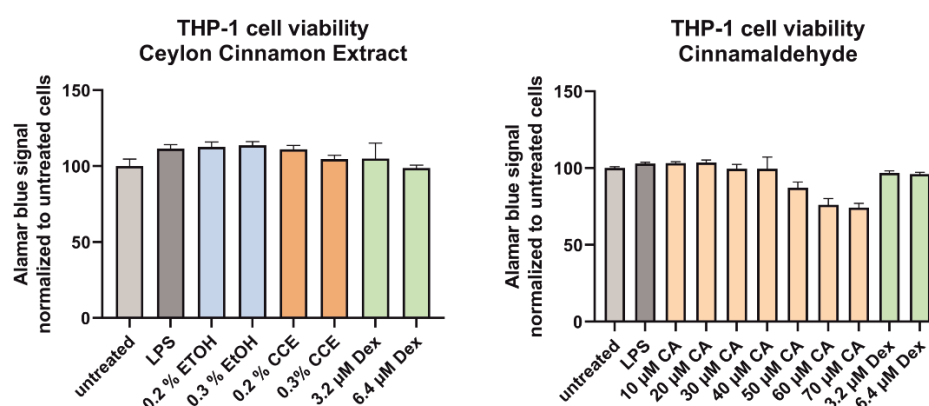


Figure 2 Cell viability. THP-1 monocytes were incubated with Ceylon cinnamon extract (CCE) at final concentrations of 0.2% and 0.3%, equivalent ethanol concentrations,

dexamethasone (Dex), or with various concentrations of cinnamaldehyde (CA). After 2 h, cells were challenged or not with LPS in a final concentration of 50 ng/mL and incubated for 4 h. Thereafter, the alamarBlue™ assay was performed to determine cell viability. Arithmetic mean values and standard deviations based on four measurement for each column; both experiments were repeated with very similar results on different days.

To evaluate toxicity, an alamarBlue™ assay was performed to determine cell viability (Fig. 2). Ceylon cinnamon extracts at concentrations of 0.2% and 0.3% elicited no toxic effects in THP-1 cells, which is in accordance with previous results (26). At concentrations of up to 40 µM, cinnamaldehyde showed no toxic effects in THP-1 monocytes, whereas concentrations of CA of 50 µM and higher resulted in reduced cell viability. Dexamethasone concentrations of 3.2 µM and 6.4 µM elicited no cytotoxic effects.

Expression of cytokine genes

We next tested the efficacy of CCE and CA in inhibiting IL-1β, IL-8, and TNF mRNA expression and IL-8 protein release in THP-1 monocytes (Fig. 3, Fig. 4). In previous studies, we determined that a concentration of 0.3% CCE is most effective in THP-1 cells (26, 27). To confirm dose dependency, we also included a concentration of 0.2% CCE in the mRNA expression experiments. For CA, we tested concentrations of 20 µM, 30 µM, 40 µM, and 50 µM CA. We compared CCE and CA with two concentrations of dexamethasone (3.2 µM and 6.4 µM) as a reference standard anti-inflammatory drug.

Inhibition of cytokine mRNA expression by CCE

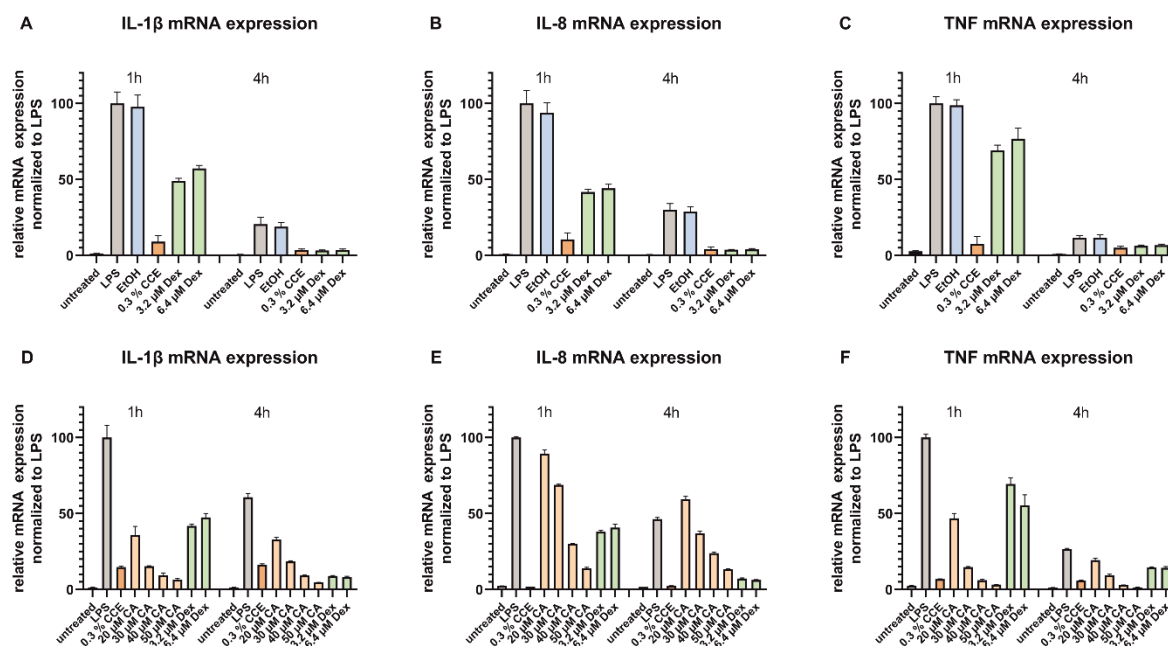


Fig. 3 Effects of Ceylon cinnamon extract (CCE) and cinnamaldehyde (CA) on

cytokine mRNA expression. Relative IL-1 β , IL-8, and TNF mRNA abundance after

different treatments, normalized to LPS stimulation. Cells were pre-incubated with 0.3%

CCE, different concentrations of CA or dexamethasone (3.2 μ M and 6.4 μ M) for 2 h. Then,

LPS (final conc. 50 ng/mL) was added and cells were incubated for a further 1 h or 4 h.

mRNA was isolated and quantified by qPCR. Arithmetic mean values and standard

deviations of two independent experiments performed on different days as triplicates,

measured in technical duplicate.

The mRNA expression of all tested NF- κ B-regulated cytokines was strongly enhanced 1 h

after stimulation with 50 ng/mL LPS with sharp decreases in the induction by 4 h (Fig. 3).

The used ethanol concentrations had no impact on gene expression. Treatment with 0.3%

CCE drastically inhibited the LPS-stimulated increases in the mRNA expression of all three

cytokines. In contrast, the inhibitory effects of 3.2 μ M and 6.4 μ M dexamethasone after 1 h

were relatively lower, but similar to CCE after 4 h. This suggests that the effects of

dexamethasone on inflammatory signaling pathways are not immediate (36).

Cinnamaldehyde showed a clear dose dependence in reducing the mRNA expression of all three cytokines examined (Fig. 3). This was expected, since the cinnamaldehyde in CCE interferes with TLR4 receptor dimerization and thus inhibits the activation of NF- κ B by LPS (28). This can explain the faster action of cinnamaldehyde compared to dexamethasone, because inflammatory TLR4 signaling is blocked already outside the cell. As shown in Figure 2, a CA concentration of 50 μ M elicited cytotoxic effects, which additionally influences gene expression. Concentrations of 30 μ M CA and 3.2 μ M dexamethasone were similarly potent in suppressing inflammatory signaling. In these experiments, the effect of 30 μ M CA on IL-1 β and TNF expression was comparable to that of CCE, but the effect was less pronounced for IL-8. Overall, whole CCE seems to be more efficient than cinnamaldehyde in the suppression of mRNA expression of inflammatory genes. We also observed this phenomenon in a previous study; one possible explanation might lie in the synergistic effects of CA with *p*-cymene, cinnamyl alcohol, and cinnamic acid, which are additional active compounds of CCE (27).

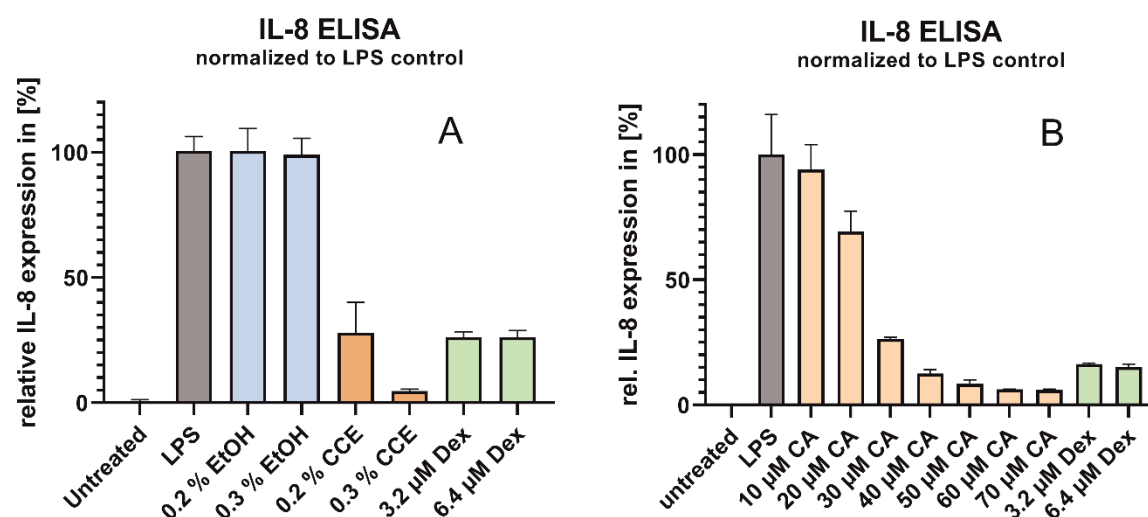


Fig 4 IL-8 protein expression. Relative protein expression of IL-8 in THP-1 cells challenged for 4 h with 50 ng/mL LPS and treated with CCE, equivalent concentration of ethanol (A), CA, or dexamethasone (B). Cells were pre-incubated with the respective treatment for 2 h prior to LPS challenge. Arithmetic mean values and standard deviations of two independent

experiments performed on different days as biological duplicates, measured in technical duplicate.

For quantification of the effects of CCE, CA, and dexamethasone on IL-8 cytokine release, THP-1 monocytes were pre-incubated with 0.2% or 0.3% CCE, equivalent concentrations of ethanol, or with 3.2 μ M or 6.4 μ M dexamethasone (Fig. 4A), and seven different concentrations of CA (Fig. 4B). LPS was added in a final concentration of 50 ng/mL, and the cells were incubated for a further 4 h. Supernatants were collected and analyzed by IL-8 ELISA. The baseline IL-8 expression of untreated cells was set to zero, while the IL-8 expression of the LPS-treated cells was set to 100% in both experimental settings. The addition of 0.2% and 0.3% ethanol had no effect on IL-8 protein expression. CCE in a concentration of 0.2% had a similar effect to 3.2 μ M or 6.4 μ M dexamethasone (Fig 4A). CCE at a concentration of 0.3% was more effective for the suppression of IL-8 than either of the dexamethasone concentrations used. CA at concentrations of 30 μ M and 40 μ M inhibited TLR4-dependent IL-8 expression to an extent comparable to the dexamethasone concentrations used (Fig. 4B).

Taken together, CCE and CA can dampen the activation of TLR4-dependent pathways during inflammation. This suggests that cinnamon compounds may also reduce the ROS/RNS-stimulated, DAMP-mediated activation of TLR4 during severe inflammation (Fig. 1).

In vitro angiogenesis assay

Reactive angiogenesis is another complication present in SARS-CoV-2-infected organs in COVID-19. The tube formation assay in HUVEC cells is a common cellular model for angiogenesis (37). We carried out preliminary tube formation assay experiments *in vitro* to determine whether CCE can also mitigate this complication of COVID-19.

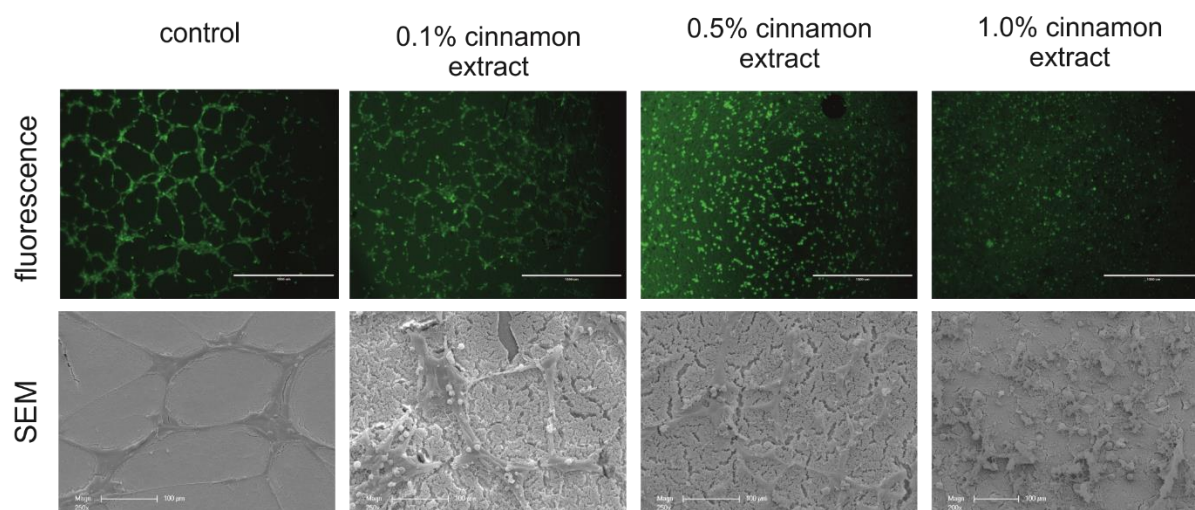


Fig 5 *In vitro* formation of tubes. HUVEC cells were grown on solubilized basement membrane extract; fluorescence light microscopy (Fig. 5A–5D). After seeding 1×10^5 cells per well in 400 μ L of medium, tube formation was recorded over the course of 6 h. Where indicated, the cells were inspected at time 0 (seeding), after 3.5 h, and 6 h. Prior to microscopic inspection, the cells were stained with calcein-AM and inspected under fluorescence light; A: control; B: 0.1% Ceylon cinnamon extract (CCE); C: 0.5% CCE; D: 1.0% CCE. Fig. 5E–5H: HUVEC cell formation at higher magnification after a total incubation period of 16 h; analysis by scanning electron microscopy (SEM). In contrast to controls, 0.1% CCE resulted in an attenuation of tube formation, whereas 0.5% and 1.0% CCE elicited cytotoxic effects in HUVEC cells.

If HUVEC cells are cultivated onto solubilized and subsequently solidified basement membrane extract matrix, they start to form a tube-like network (Fig. 5A). This morphogenetic pattern is caused by a sprouting of endothelial cells, which is routinely used as a surrogate for angiogenesis. The temporal analysis of this morphogenetic process by fluorescence light microscopy revealed an inhibitory effect of 0.1% CCE after 3.5 h and 6 h hours (Fig. 5B), whereas higher dosages (0.5% and 1.0% cinnamon, Fig. 5C and 5D) showed direct cytotoxic effects. The analysis of this sprouting process by scanning electron

microscopy (SEM) showed an altered cell morphology in response to 0.1% CCE (Fig. 5F). Higher dosages resulted in cellular damage and apoptosis (Fig. 5G and 5H). The inhibition of angiogenesis by CCE and CA is well documented in the literature (38-43). Suprisingly one studie find a angigenesis promoting effect for CA in the context of wouldhealing (44).

Side effects of corticosteroids

Despite the clinical efficacy of dexamethasone for COVID-19 patients, unwanted side-effects are known for corticosteroids, e.g. steroid psychosis (45), hyperglycemia, especially in diabetic patients (46), and secondary bacterial pneumonia or invasive fungal infection from immunosuppression (47). Hypothetically, cinnamon or cinnamaldehyde should act synergistically with dexamethasone. A combination of both may thus allow the administration of a reduced dosage of dexamethasone. As shown in an animal model, the concurrent intake of cinnamon can mitigate the side-effects of dexamethasone (48). Cinnamon can also positively influence insulin resistance, lipid metabolism, and glucose transport (48, 49).

Dosage form and dosage

Ethanolc cinnamon extract is much more suitable than cinnamon powder for cell culture experiments, since, in contrast to the powder form, it can be applied quantitatively by pipetting directly into the culture medium. In contrast, the majority of clinical studies on cinnamon have used encapsulated cinnamon bark powder (30). Since almost no reports describing ethanolc CCE for human use have been published to date, future studies on COVID-19 patients should use the encapsulated powder. Typically, doses of encapsulated cinnamon vary between 500 mg and 3 g per day for adults (50-54). However, to the best of our knowledge, no study administering cinnamon alone or in combination with dexamethasone for COVID-19 has been published to date. Therefore, clinical studies are needed before the treatment of COVID-19 patients with cinnamon or its compounds can be recommended. The Ceylon cinnamon should be of pharmaceutical grade as described here.

Since dexamethasone and cinnamon act to dampen inflammation via distinct pathways, we suggest that these compounds are likely to have synergistic effects when administered concomitantly.

Conclusions

Cinnamon has been used as a medicine for thousands of years in traditional medical practices (55). Its anti-inflammatory effects are well documented. The inhibition of TLR4 dimerization, in particular, is an important anti-inflammatory mechanism (28). During the so called 'cytokine storm', not only cytokines but also ROS/RNS and DAMPs contribute to amplification of inflammation (56). Our results indicate that dexamethasone, which is now being used to dampen excessive inflammation in COVID-19, could be combined with a Ceylon cinnamon preparation. In contrast to dexamethasone, which inhibits the activity of the pro-inflammatory transcription factor NF- κ B (22), cinnamon can suppress the secondary activation of TLR4 by DAMPs (28). These differences in modes of action suggest the possibility of synergistic effects. Further studies may pave the way towards clinical studies of cinnamon derivatives for the treatment of COVID-19.

Our results suggest that intake of Ceylon cinnamon in combination with dexamethasone may prevent the 'cytokine storm' and help to reduce the dosage of dexamethasone and mitigate its side effects. Preliminary results indicate that cinnamon may also reduce angiogenesis. Note, however, that Ceylon cinnamon should not be substituted by Cassia cinnamon, which may comprise high amounts of liver-toxic coumarin (31).

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

376 **Author contributions:** KL, AM, ALL and UP designed and conducted research; KL, AM,
377 ALL and JF-N analyzed data; KL, AM, ALL, JF-N, UP and WL wrote and edited the
378 manuscript; KL, AM, JF-N and UP had primary responsibility for final content.

379 References

- 380 1. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary
381 Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. *The New England journal of*
382 *medicine*. 2020;383(2):120-8.
- 383 2. Heneka MT, Golenbock D, Latz E, Morgan D, Brown R. Immediate and long-term
384 consequences of COVID-19 infections for the development of neurological disease. *Alzheimer's*
385 *research & therapy*. 2020;12(1):69.
- 386 3. Mitrani RD, Dabas N, Goldberger JJ. COVID-19 cardiac injury: Implications for long-term
387 surveillance and outcomes in survivors. *Heart rhythm*. 2020;17(11):1984-90.
- 388 4. Berlin DA, Gulick RM, Martinez FJ. Severe Covid-19. *The New England journal of medicine*.
389 2020;383(25):2451-60.
- 390 5. Lucas K, Rosch M, Langguth P. Molecular hydrogen (H₂) as a potential treatment for acute
391 and chronic fatigue. *Archiv der Pharmazie*. 2020:e2000378.
- 392 6. Hu B, Huang S, Yin L. The cytokine storm and COVID-19. *Journal of medical virology*. 2020.
- 393 7. Ye Q, Wang B, Mao J. The pathogenesis and treatment of the 'Cytokine Storm' in COVID-19.
394 *The Journal of infection*. 2020;80(6):607-13.
- 395 8. Cauchois R, Koubi M, Delarbre D, Manet C, Carvelli J, Blasco VB, et al. Early IL-1 receptor
396 blockade in severe inflammatory respiratory failure complicating COVID-19. *Proceedings of the*
397 *National Academy of Sciences of the United States of America*. 2020;117(32):18951-3.
- 398 9. Han H, Ma Q, Li C, Liu R, Zhao L, Wang W, et al. Profiling serum cytokines in COVID-19
399 patients reveals IL-6 and IL-10 are disease severity predictors. *Emerging microbes & infections*.
400 2020;9(1):1123-30.
- 401 10. Takahashi T, Ellingson MK, Wong P, Israelow B, Lucas C, Klein J, et al. Sex differences in
402 immune responses that underlie COVID-19 disease outcomes. *Nature*. 2020;588(7837):315-20.
- 403 11. Liu T, Zhang L, Joo D, Sun S-C. NF-κB signaling in inflammation. *Signal Transduction and*
404 *Targeted Therapy*. 2017;2(1):17023.
- 405 12. Rasmussen MK, Iversen L, Johansen C, Finnemann J, Olsen LS, Kragballe K, et al. IL-8 and p53
406 are inversely regulated through JNK, p38 and NF-κB p65 in HepG2 cells during an inflammatory
407 response. *Inflammation Research*. 2008;57(7):329-39.
- 408 13. Venereau E, Casalgrandi M, Schiraldi M, Antoine DJ, Cattaneo A, De Marchis F, et al.
409 Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory cytokine
410 release. *The Journal of experimental medicine*. 2012;209(9):1519-28.
- 411 14. Jakovac H. COVID-19 and hypertension: is the HSP60 culprit for the severe course and worse
412 outcome? *American journal of physiology Heart and circulatory physiology*. 2020;319(4):H793-h6.
- 413 15. Andersson U, Ottestad W, Tracey KJ. Extracellular HMGB1: a therapeutic target in severe
414 pulmonary inflammation including COVID-19? *Molecular medicine (Cambridge, Mass)*.
415 2020;26(1):42.
- 416 16. Street ME. HMGB1: A Possible Crucial Therapeutic Target for COVID-19? *Hormone research*
417 *in paediatrics*. 2020;93(2):73-5.
- 418 17. Chen R, Huang Y, Quan J, Liu J, Wang H, Billiar TR, et al. HMGB1 as a potential biomarker and
419 therapeutic target for severe COVID-19. *Heliyon*. 2020;6(12):e05672.

18. Chen L, Long X, Xu Q, Tan J, Wang G, Cao Y, et al. Elevated serum levels of S100A8/A9 and HMGB1 at hospital admission are correlated with inferior clinical outcomes in COVID-19 patients. *Cellular & molecular immunology*. 2020;17(9):992-4.
19. Ziegler K, Kunert AT, Reinmuth-Selzle K, Leifke AL, Widera D, Weller MG, et al. Chemical modification of pro-inflammatory proteins by peroxyxynitrite increases activation of TLR4 and NF- κ B: Implications for the health effects of air pollution and oxidative stress. *Redox biology*. 2020;101581.
20. Blaser H, Dostert C, Mak TW, Brenner D. TNF and ROS Crosstalk in Inflammation. *Trends in cell biology*. 2016;26(4):249-61.
21. Lucas K, Maes M. Role of the Toll Like receptor (TLR) radical cycle in chronic inflammation: possible treatments targeting the TLR4 pathway. *Molecular neurobiology*. 2013;48(1):190-204.
22. Krieger S, Sorrells SF, Nickerson M, Pace TW. Mechanistic insights into corticosteroids in multiple sclerosis: war horse or chameleon? *Clinical neurology and neurosurgery*. 2014;119:6-16.
23. Greulich F, Wierer M, Mechtidou A, Gonzalez-Garcia O, Uhlenhaut NH. The glucocorticoid receptor recruits the COMPASS complex to regulate inflammatory transcription at macrophage enhancers. *Cell reports*. 2021;34(6):108742.
24. Tomazini BM, Maia IS, Cavalcanti AB, Berwanger O, Rosa RG, Veiga VC, et al. Effect of Dexamethasone on Days Alive and Ventilator-Free in Patients With Moderate or Severe Acute Respiratory Distress Syndrome and COVID-19: The CoDEX Randomized Clinical Trial. *Jama*. 2020;324(13):1307-16.
25. Lucas K, Morris G, Anderson G, Maes M. The Toll-Like Receptor Radical Cycle Pathway: A New Drug Target in Immune-Related Chronic Fatigue. *CNS & neurological disorders drug targets*. 2015;14(7):838-54.
26. Schink A, Neumann J, Leifke AL, Ziegler K, Fröhlich-Nowoisky J, Cremer C, et al. Screening of herbal extracts for TLR2- and TLR4-dependent anti-inflammatory effects. *PloS one*. 2018;13(10):e0203907.
27. Schink A, Naumoska K, Kitanovski Z, Kampf CJ, Fröhlich-Nowoisky J, Thines E, et al. Anti-inflammatory effects of cinnamon extract and identification of active compounds influencing the TLR2 and TLR4 signaling pathways. *Food & function*. 2018;9(11):5950-64.
28. Youn HS, Lee JK, Choi YJ, Saitoh SI, Miyake K, Hwang DH, et al. Cinnamaldehyde suppresses toll-like receptor 4 activation mediated through the inhibition of receptor oligomerization. *Biochemical pharmacology*. 2008;75(2):494-502.
29. Nayak IN, Chinta R, Jetty R. Anti-Atherosclerotic Potential of Aqueous Extract of Cinnamomum Zeylanicum Bark against Glucocorticoid Induced Atherosclerosis in Wistar Rats. *Journal of clinical and diagnostic research : JCDR*. 2017;11(5):Fc19-fc23.
30. Lucas K, Fröhlich-Nowoisky J, Oppitz N, Ackermann M. Cinnamon and hop extracts as potential immunomodulators for severe COVID-19 cases. *Front Plant Sci*. 2021.
31. Wang YH, Avula B, Nanayakkara NP, Zhao J, Khan IA. Cassia cinnamon as a source of coumarin in cinnamon-flavored food and food supplements in the United States. *Journal of agricultural and food chemistry*. 2013;61(18):4470-6.
32. Menacher G, Steinritz D, Schmidt A, Popp T, Worek F, Gudermann T, et al. Effects of anti-inflammatory compounds on sulfur mustard injured cells: Recommendations and caveats suggested by in vitro cell culture models. *Toxicology letters*. 2018;293:91-7.
33. Piehler AP, Grimholt RM, Ovstebø R, Berg JP. Gene expression results in lipopolysaccharide-stimulated monocytes depend significantly on the choice of reference genes. *BMC immunology*. 2010;11:21.
34. DeCicco-Skinner KL, Henry GH, Cataisson C, Tabib T, Gwilliam JC, Watson NJ, et al. Endothelial cell tube formation assay for the in vitro study of angiogenesis. *Journal of visualized experiments : JoVE*. 2014(91):e51312.
35. Esch MB, Post DJ, Shuler ML, Stokol T. Characterization of in vitro endothelial linings grown within microfluidic channels. *Tissue engineering Part A*. 2011;17(23-24):2965-71.

- 470 36. Baker JB, Barsh GS, Carney DH, Cunningham DD. Dexamethasone modulates binding and
471 action of epidermal growth factor in serum-free cell culture. *Proceedings of the National Academy of*
472 *Sciences of the United States of America*. 1978;75(4):1882-6.
- 473 37. Stryker ZI, Rajabi M, Davis PJ, Mousa SA. Evaluation of Angiogenesis Assays. *Biomedicines*.
474 2019;7(2).
- 475 38. Lu J, Zhang K, Nam S, Anderson RA, Jove R, Wen W. Novel angiogenesis inhibitory activity in
476 cinnamon extract blocks VEGFR2 kinase and downstream signaling. *Carcinogenesis*. 2010;31(3):481-
477 8.
- 478 39. Bansode RR, Leung T, Randolph P, Williams LL, Ahmedna M. Cinnamon extract inhibits
479 angiogenesis in zebrafish and human endothelial cells by suppressing VEGFR1, VEGFR2, and PKC-
480 mediated MAP kinase. *Food science & nutrition*. 2013;1(1):74-82.
- 481 40. Bae WY, Choi JS, Kim JE, Jeong JW. Cinnamic aldehyde suppresses hypoxia-induced
482 angiogenesis via inhibition of hypoxia-inducible factor-1 α expression during tumor progression.
483 *Biochemical pharmacology*. 2015;98(1):41-50.
- 484 41. Hamidpour R, Hamidpour M, Hamidpour S, Shahlari M. Cinnamon from the selection of
485 traditional applications to its novel effects on the inhibition of angiogenesis in cancer cells and
486 prevention of Alzheimer's disease, and a series of functions such as antioxidant, anticholesterol,
487 antidiabetes, antibacterial, antifungal, nematocidal, acaracidal, and repellent activities. *Journal of*
488 *traditional and complementary medicine*. 2015;5(2):66-70.
- 489 42. Zhang K, Han ES, Dellinger TH, Lu J, Nam S, Anderson RA, et al. Cinnamon extract reduces
490 VEGF expression via suppressing HIF-1 α gene expression and inhibits tumor growth in mice.
491 *Molecular carcinogenesis*. 2017;56(2):436-46.
- 492 43. Patra K, Jana S, Sarkar A, Mandal DP, Bhattacharjee S. The inhibition of hypoxia-induced
493 angiogenesis and metastasis by cinnamaldehyde is mediated by decreasing HIF-1 α protein synthesis
494 via PI3K/Akt pathway. *BioFactors (Oxford, England)*. 2019;45(3):401-15.
- 495 44. Yuan X, Han L, Fu P, Zeng H, Lv C, Chang W, et al. Cinnamaldehyde accelerates wound
496 healing by promoting angiogenesis via up-regulation of PI3K and MAPK signaling pathways.
497 *Laboratory investigation; a journal of technical methods and pathology*. 2018;98(6):783-98.
- 498 45. Berthelot JM, Le Goff B, Maugars Y. Side effects of corticosteroid injections: what's new?
499 *Joint bone spine*. 2013;80(4):363-7.
- 500 46. Polderman JAW, Farhang-Razi V, Van Dieren S, Kranke P, DeVries JH, Hollmann MW, et al.
501 Adverse side effects of dexamethasone in surgical patients. *Cochrane Database of Systematic*
502 *Reviews*. 2018(11).
- 503 47. Ni YN, Chen G, Sun J, Liang BM, Liang ZA. The effect of corticosteroids on mortality of
504 patients with influenza pneumonia: a systematic review and meta-analysis. *Critical care (London,*
505 *England)*. 2019;23(1):99.
- 506 48. Sheng X, Zhang Y, Gong Z, Huang C, Zang YQ. Improved Insulin Resistance and Lipid
507 Metabolism by Cinnamon Extract through Activation of Peroxisome Proliferator-Activated
508 Receptors. *PPAR Research*. 2008;2008:581348.
- 509 49. Cao H, Graves DJ, Anderson RA. Cinnamon extract regulates glucose transporter and insulin-
510 signaling gene expression in mouse adipocytes. *Phytomedicine : international journal of*
511 *phytotherapy and phytopharmacology*. 2010;17(13):1027-32.
- 512 50. Khan A, Safdar M, Ali Khan MM, Khatkhat KN, Anderson RA. Cinnamon improves glucose and
513 lipids of people with type 2 diabetes. *Diabetes care*. 2003;26(12):3215-8.
- 514 51. Zare R, Nadjarzadeh A, Zarshenas MM, Shams M, Heydari M. Efficacy of cinnamon in
515 patients with type II diabetes mellitus: A randomized controlled clinical trial. *Clinical nutrition*
516 *(Edinburgh, Scotland)*. 2019;38(2):549-56.
- 517 52. Zareie A, Sahebkar A, Khorvash F, Bagherniya M, Hasanzadeh A, Askari G. Effect of cinnamon
518 on migraine attacks and inflammatory markers: A randomized double-blind placebo-controlled trial.
519 *Phytotherapy research : PTR*. 2020;34(11):2945-52.

- 520 53. Talaei B, Amouzegar A, Sahranavard S, Hedayati M, Mirmiran P, Azizi F. Effects of Cinnamon
521 Consumption on Glycemic Indicators, Advanced Glycation End Products, and Antioxidant Status in
522 Type 2 Diabetic Patients. *Nutrients*. 2017;9(9).
- 523 54. Mirmiran P, Davari M, Hashemi R, Hedayati M, Sahranavard S, Bahreini S, et al. A
524 randomized controlled trial to determining the effect of cinnamon on the plasma levels of soluble
525 forms of vascular adhesion molecules in type 2 diabetes mellitus. *European journal of clinical*
526 *nutrition*. 2019;73(12):1605-12.
- 527 55. Pan SY, Litscher G, Gao SH, Zhou SF, Yu ZL, Chen HQ, et al. Historical perspective of
528 traditional indigenous medical practices: the current renaissance and conservation of herbal
529 resources. *Evidence-based complementary and alternative medicine : eCAM*. 2014;2014:525340.
- 530 56. Kumar V. Toll-like receptors in sepsis-associated cytokine storm and their endogenous
531 negative regulators as future immunomodulatory targets. *International immunopharmacology*.
532 2020;89(Pt B):107087.

533