Modifications of IL-6 by Hypochlorous Acids: Effects on Receptor Binding

Lori I. Robins,* Erika K. Keim, Deborah B. Robins, John S. Edgar, John S. Meschke, Philip R. Gafken, and Jeffrey F. Williams

ABSTRACT: Interleukin-6 (IL-6) has been implicated in the pathogenesis of inflammatory events including those seen with COVID-19 patients. Positive clinical responses to monoclonal antibodies directed against IL-6 receptors (IL-6Rs) suggest that interference with IL-6-dependent activation of pro-inflammatory pathways offers a useful approach to therapy. We exposed IL-6 to hypochlorous acid (HOCl) in vitro at concentrations reported to develop in vivo. After HOCl treatment, binding of IL-6 to IL-6R was reduced in a dose-dependent manner using a bioassay with human cells engineered to provide a luminescence response to signal transduction upon receptor activation. Similar results followed the exposure of IL-6 to N-chlorotaurine (NCT) and hypobromous acid (HOBr), two other reactive species produced in vivo. SDS-PAGE analysis of HOCl-treated IL-6 showed little to no fragmentation or aggregation up to 1.75 mM HOCl, suggesting that the modifications induced at concentrations below 1.75 mM took place on the intact protein. Mass spectrometry of trypsin-digested fragments identified oxidative changes to two amino acid residues, methionine 161 and tryptophan 157, both of which have been implicated in receptor binding of the cytokine. Our findings suggest that exogenous HOCl and NCT might bring about beneficial effects in the treatment of COVID-19. Further studies on how HOCl and HOBr and their halogenated amine derivatives interact with IL-6 and related cytokines in vivo may open up alternative therapeutic interventions with these compounds in COVID-19 and other hyperinflammatory diseases.

INTRODUCTION

Interleukin-6 (IL-6) has long been associated with chronic inflammatory diseases such as rheumatoid arthritis where levels of IL-6 are elevated in the serum and synovial fluids.1,2 IL-6 has now been implicated in the pathogenesis of pulmonary and systemic lesions resulting from the “cytokine storm” in seriously afflicted COVID-19 patients.3,4 Elevated levels of IL-6 have also been identified as biomarkers for disease severity and are associated with hyperinflammation.5,6 Currently, monoclonal antibodies directed against IL-6Rs are used to treat inflammatory diseases.7−9 There is now compelling evidence of therapeutic benefits arising from administration of these same monoclonal antibody products as part of the clinical management of SARS-CoV-2 infection, particularly if the intervention is used in patients with severe disease and early during the treatment.10,11 These observations make a case for IL-6-mediated events being causal in the most important and often terminal consequences of COVID-19 and point to the need for mitigation of these reactions in the treatment and resolution of SARS-CoV-2 infections. While parenteral IL-6R monoclonal antibody products may provide proven clinical benefits, their routine use is not realistic for this purpose in many healthcare systems around the world. They are expensive, their use requires hospitalization of patients, and they can cause adverse reactions that need medical attention.10,12

HOCl, produced by myeloperoxidase (MPO), is part of the innate immune response in vivo. It functions beneficially as the first line of chemical defense against invading pathogens but can also participate in the pathogenesis of certain chronic inflammatory disease processes.12,13 The expression of inflammatory cytokines, including IL-6, is known to be affected by HOCl and NCT.14−17 Exogenous HOCl, applied topically, brings about beneficial changes that aid in the healing and resolution of injured and infected tissues.18−20

© 2021 The Authors. Published by American Chemical Society
Here, we demonstrate rapid modification of IL-6 in vitro upon exposure to HOCl and two other halogen-containing compounds produced in vivo, NCT and hypobromous acid (HOBr). Markedly impaired IL-6R binding was demonstrated using engineered human cells that luminesce following activation by IL-6. Mass spectrometry analysis of trypsin-digested fragments identified the oxidation of methionine and tryptophan residues that are involved with IL-6 receptor binding. The results suggest that hypohalous acids and corresponding halogenated amines may have direct inhibitory effects on pro-inflammatory mediators such as IL-6. They also suggest that it may be worthwhile exploring this mechanism in other systemic inflammatory pathological processes involving IL-6.

**METHODS AND MATERIALS**

Reagents for the iodometric titrations were purchased from Hach (dissolved oxygen 3 powder pillows, potassium iodide powder pillows, sodium thiosulfate digital titrator cartridge (0.113 N), and starch indicator solution). Sodium chloride (NaCl), sodium hydroxide (NaOH), ELISA wash buffer (0.1 M), sodium chloride (NaCl), sodium hydroxide (NaOH), ELISA wash buffer (50-184-79), ELISA stop buffer (SS03), and sodium thiosulfate (Na2S2O3, STS) were purchased from Fisher Scientific. Sodium bromide and taurine were purchased from ACROS Organics. Water from a MilliQ water purification system was used for all experiments. HOCl (pH 4) was provided by BioRad Inc. Human IL-6 was purchased from GenScript, 2800 Woods Hollow Road Madison, WI 53711, USA (JA2501).

**HOCl Concentration Determination.** Sodium hydroxide (1 M) was added to a solution of HOCl to increase pH to 10. The UV–Vis absorbance of the sample was measured at 292 nm, and the concentration was determined with an extinction coefficient of 350 M⁻¹ cm⁻¹.²⁷

**Conversion of HOCl to HOBr.** The conversion of HOCl to HOBr was achieved by the addition of NaBr (1.1 equiv) to HOCl at pH 4. The conversion was monitored by a UV–Vis spectrometer (BioMate 3S) and the absorbance of HOCl at 236 nm shifted to 260 nm after the addition of NaBr. HOBr was monitored at 260 nm with an extinction coefficient of 160 M⁻¹ cm⁻¹.²⁸

**Synthesis of N-Chlorotaurine.** The synthesis of NCT was done following published procedures.²⁹,³⁰ Briefly, HCl was diluted with 1 M NaOH to convert HOCl to HOCl. Taurine (10 molar equiv) was added to the solution in four aliquots with a 1 min interval between each addition. The absorbance was measured at 250 nm with an extinction coefficient of 397 M⁻¹ cm⁻¹.³¹ The pH was adjusted to neutral using 5 M HCl prior to use.

**ELISA Assay.** The Invitrogen human IL-6 ELISA kit (catalog # 88–7066) was purchased and used according to the provided protocol. Briefly, 96-well plates were coated with the capture antibody (anti-human IL-6 antibody).

**IL-6 (2.5 μg/mL) Treated with HOCl.** IL-6 (2.5 μg/mL) was incubated with HOCl (0–72 μM) for 5 min and then quenched with 1% STS. The detection for human IL-6 antibody conjugated with HRP was used to detect the antigen. The samples were diluted to 1:1,000,000 and run in triplicate. All experiments were done a minimum of three times. Graphs include averages and standard deviations. One-way ANOVA with post-hoc Dunnett’s test (α = 0.05) was conducted on untreated and HOCl and HOBr-treated IL-6 in high and low-concentration experiments to test for statistical significance using GraphPad Prism.

**Tryptsin-Digested IL-6 Peptide Analysis by Mass Spectrometry.** IL-6 and treated IL-6 samples were prepared following the rapid digestion-trypsin protocol (Promega, VA1060). Three HOCl-treated samples were analyzed, single analyses were completed on HOBr- and NCT-treated samples, and two control samples were analyzed. Prior to digestion, the IL-6-treated samples were prepared by incubating 25 μL of 1 mg/mL IL-6 with 25 μL of 3.43 mM HOCl, HOBr, or NCT. After 5 min of incubation with the active halogen compound, the solution was quenched with 5 μL of 1% STS. The control sample was prepared by adding 25 μL of 1 mg/mL IL-6 with 25 μL of water. Tandem mass spectrometry was performed on HOCl-treated samples and an IL-6 control using a Thermo Orbitrap mass spectrometer coupled to a Waters Acquity UPLC system. Separations were performed on a Waters CSH 1.0 × 100 mm column. All data analyses were performed in PMI-Byonic where all potential modifications were searched. Additional mass spectrometry studies on HOCl- and HOBr- and NCT-treated samples were carried out on a Thermoscientific Orbitrap Fusion mass spectrometer coupled to a ThermoScientific Easy nLC-1000 HPLC system. Chromatographic separations were performed using a home-built 75 μm × 25 cm capillary column packed with a ReproSil 3 μm particle size C18 material. Data were analyzed with Proteome Discoverer v2.4, a protein database made up of a combination of human (Uniprot UP000005640, downloaded 12-01-19) and common contaminants (cRAP) databases and the recombinant human IL-6 protein sequence. Dynamic modifications were set to mono- and di-oxidation of methionine and tryptophan (+15.995 and +31.990 Da, respectively), acetylation of the N-terminus (+42.011 Da), loss of N-terminus methionine (−131.040 Da), and loss of N-terminus methionine plus acetylation of the N-terminus (−89.030 Da) and static modification of carbamidomethylation of cysteine (+57.021 Da) as this included the modifications determined by the initial studies described above. A fixed value PSM validator was used for peptide validation.

**SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE).**

**Sample Preparation for IL-6 Dose-Response with HOCl.** IL-6 (0.1 mg/mL) was incubated with HOCl (0–6 mM) for 5 min. The samples were then quenched with 1 μL of 1% STS. The control sample contained IL-6 (0.1 mg/mL) in water. The samples were treated with Laemmli sample buffer (Biorad 1610747) and heated at 95 °C for 10 min. The samples were then electrophoresed on a 12% acrylamide gel (Biorad 4561043) and stained with Coomassie (Biorad 1610786).

**Sample Preparation for IL-6 with HOCl and HOBr.** IL-6 (0.1 mg/mL) was incubated with 1.7 mM HOCl or 1.7 mM HOBr for 5 min. The samples were then quenched with 1 μL of 1% STS. The control sample contained IL-6 (0.16 mg/mL) in water. The samples were prepared and electrophoresed as described above.
**IL-6 Bioassay.** The IL-6 bioassay kit from Promega (JA2501) was used according to the provided protocols. Each experiment was incubated for 6 h at 37 °C prior to luminescence readings. All samples and combinations were run in at least two separate experiments with at least two replicates and read on a Molecular Devices SpectraMax iD3 multi-mode plate reader. The average and standard deviations are shown on the graphs.

**HOCl-, HOBr-, and NCT-Treated IL-6 Bioassays.** HOCl-treated IL-6 was prepared by incubating 3 μg/mL IL-6 with 18 μM–2.7 mM HOCl for 5 min prior to incubation with bioassay cells. HOBr-treated IL-6 was prepared by incubating 3 μg/mL IL-6 with 2.2 mM HOBr for 5 min. NCT-treated IL-6 was prepared by treating 3 μg/mL IL-6 with 2.2 mM NCT for 15 min. All HOCl-, HOBr-, and NCT-treated IL-6 samples and IL-6 control samples were serially diluted threefold in RPMI-1640 containing 10% FBS prior to application of cultured cells in the IL-6 bioassay. This quenched the remaining active halogens prior to the assay and was confirmed by negative tests for active chloride (HACH 2745050). The starting concentration of IL-6 and HOCl-, HOBr-, or NCT-treated IL-6 was 3 μg/mL and was serially diluted threefold. The final concentrations in the presence of the cells ranged from 0.015 to 1 μg/mL. Two-way ANOVA statistical analyses with Dunnett’s multiple comparison (family-wise, α = 0.05) of HOCl, HOBr, and NCT treatments compared across treatment and concentration were conducted on the luminescence response from the IL-6 bioassay using GraphPad Prism.

**Inhibition of IL-6R Bioassay.** IL-6 inhibition was assayed by serially diluting 50 μg/mL anti-IL-6 receptors (ThermoFisher AHR0061) threefold and incubating it with the IL-6 bioassay cells at 37 °C for 20 min. Untreated native IL-6 (1 μg/mL, 12.5 μL) was then added to the cells to test for inhibition.

**IL-6 Binding Studies.** IL-6 binding studies were accomplished by incubating serial dilutions of HOCl-, HOBr-, and NCT-treated IL-6 with concentrations ranging from 0.015 to 1 μg/mL for 30 min at 37 °C. IL-6 (0.1 μg/μL, 12.5 μL) was then added to the cells to test for activity.

**RESULTS**

IL-6 was incubated with various concentrations of HOCl to determine if monoclonal antibody binding was affected by HOCl treatment in an ELISA assay. A decrease in IL-6 binding was seen at all concentrations tested (Figure 1A). Similar results were seen with HOBr (Figure 1B). Higher concentrations of IL-6 and 0–72 μM HOCl were incubated prior to ELISA analysis to determine the dose response for known intact IL-6 (see Figure 2); binding was reduced by ~50% at 72 μM HOCl (Figure 1C).

An in vitro assay with human cells engineered to express the IL-6 receptor with a luciferase reporter signal as an endpoint of receptor activation was used to determine if HOCl-induced modifications affected the response. Unmodified IL-6 induced signal transduction measured in relative luminescence units (RLU). A decrease in signal transduction was seen as the concentration of HOCl was increased from 18 to 143 μM (Figure 2A). Little to no signal transduction was seen at 143 μM. At higher concentrations of HOCl (0.26 and 2.6 mM), NCT (2.2 mM), and HOBr (2.2 mM), signal transduction after incubation with IL-6 was not detected (Figure 2B). Binding of IL-6 to the IL-6 receptor decreased in a concentration-dependent manner after the engineered cells were pre-incubated with an anti-IL-6R antibody (Figure 2C).

**Figure 1.** ELISA assay of HOCl- or HOBr-treated IL-6. (A) IL-6 (150 pg/mL, 7 pM) was incubated with various concentrations of HOCl ranging from 0 to 48 μM (0–6.9 × 10^-6 molar equiv) for 5 min. The results of all tested concentrations of HOCl showed a significant difference from the control (p < 0.001). (B) IL-6 (150 pg/mL, 7 pM) was incubated with various concentrations of HOBr (0–22 μM; 0–3.1 × 10^-6 molar equiv) for 5 min. The results of all concentrations of HOBr tested were significantly different from the controls (p < 0.001). (C) IL-6 (2.5 μg/mL, 119 nM) was incubated with HOCl ranging from 0 to 72 μM (0–605 molar equiv) for 5 min. The results of treatment with 72 μM HOCl were significantly different from the controls (p < 0.001).

Modified IL-6 did not act as a receptor antagonist, as demonstrated in experiments in which authentic IL-6 was added to the cells subsequent to exposure to the modified protein (Figure 2D).

The structural integrity of IL-6 was tested at various concentrations of HOCl by SDS-PAGE. Analysis showed no fragmentation or aggregation of IL-6 in the presence of up to 1000 molar equiv of HOCl (Figure 3A). At ~3500 molar equiv, the intact protein was not detected by SDS-PAGE. Concentration-dependent aggregation of IL-6 modified by exposure to HOBr was observed by SDS-PAGE (Figure 3B).

Mass spectrometry was used to determine the location of modifications to IL-6. Trypsin digestion, covering 77%–97% of the protein sequence, revealed a single fragment that was oxidized in the presence of HOCl, HOBr, and NCT (Figure 4). Met 161 and Trp 157 were oxidized in the presence of hypohalous acids and NCT (Figure 4). The overall percent modification was similar for the HOCl-treated and untreated IL-6 digest samples (~87%). The percent oxidation increased to 95% for HOBr-treated IL-6 and it was lower (78%) in the presence of NCT. A single oxidation of Met 161 and di-oxidation of Trp 157 were only seen in the presence of HOCl and NCT, indicating that although the percent modifications were similar to or less than the control, the products are different and not simply due to oxidations occurring during the digestion. HOBr oxidation ratios were significantly higher than the control in three-fifths of the identified fragments (Figure 4C).
DISCUSSION

IL-6 has emerged as a major participant in the cascade of inflammatory events that are critical to the pathogenesis of the “cytokine storm” that is often causal for death in COVID-19 patients. In our ELISA experiments, exposure of IL-6 to HOCl or HOBr led to a rapid decline in immunological reactivity with IL-6-specific monoclonal antibodies. The inhibition of binding in the ELISA assay is likely the result of modifications to the protein such as those detected by mass spectrometry at less than 1000 HOCl molar equivalents. At concentrations corresponding to higher molar equivalents of HOCl, it is possible that aggregation and/or fragmentation are responsible for the decrease in antibody binding as seen by SDS-PAGE. The structural integrity of IL-6 in the presence of high molar equivalents may be due to the local environment and the amino acid sequence of the protein.31 While many proteins fragment at lower molar equivalents of HOCl, proteins such as collagen and immunoglobulins remain intact in the presence of 200 and 800 molar equivalents, respectively.

Exposure to HOCl altered the ability of IL-6 to bind to the IL-6R and triggered the receptor-mediated generation of luminescence in the in vitro bioassay. Signal transduction induced by treated cytokine preparations showed a dose response for concentrations of HOCl ranging from 18 to 143 μM. Since cells pre-incubated with HOCl-treated IL-6 responded normally following addition of native IL-6, we infer that the HOCl-treated cytokine was no longer able to bind to the receptor. The conditions tested are consistent with...
concentrations of HOCl that might occur in vivo given that HOCl can be produced at a rate of 134 mM/min in phagosomes and is estimated to be between 25 and 50 mM at sites of inflammation.\textsuperscript{34-36}

HOCl has a short lifetime attributable to its high reactivity and rapid modification of substrates including sulfur-containing amino acids and amines.\textsuperscript{13,30,34} Taurine, abundant in human plasma and tissues, is readily chlorinated by HOCl, and NCT is well recognized as a long-lived oxidant.\textsuperscript{37-40} HOBr, in contrast, is produced in vivo by eosinophil granulocytes through a myeloperoxidase pathway that depends upon the availability of intracellular Br\textsuperscript{−} ions. The corresponding reaction product with taurine is N-bromotaurine (NBT).\textsuperscript{41} The concentrations of the two hypohalous acids (HOCl and HOBr) differ markedly in vivo due to differences in the availability of Cl\textsuperscript{−} and Br\textsuperscript{−} ions. Nonetheless, HOBr is clearly a significant factor in the reactive oxygen species involved in host tissue responses to infection and injury. Eosinophilia, for example, is associated with better clinical outcomes for COVID-19 patients, and HOBr and NBT are likely contributors.\textsuperscript{42}

Exposure of IL-6 to freshly produced HOBr and NCT led to changes similar to those seen with HOCl. These findings suggest that comparable modifications or aggregation/fragmentation of IL-6 were affected by HOCl, HOBr, and NCT. Mass spectrometry confirmed that the modifications after exposure to HOCl, HOBr, and NCT were restricted to Met 161 and Trp 157 (Figure 4). The mono- and di-oxidation products detected are common for both Met and Trp residues in the presence of HOCl.\textsuperscript{33,42} Met 161 has been implicated in IL-6 receptor binding and was shown to oxidize in the presence of chloramine T (N-chloro-p-toluenesulfonamide) in addition to other Met residues.\textsuperscript{34} Trp 157, a conserved residue near the receptor binding region, was modified by both HOCl and chloramine T. The structural integrity of IL-6 was preserved after treatment with chloramine T, which was also the case with HOCl under the experimental conditions.\textsuperscript{31} It is possible that chlorinated amines (e.g., lysine) formed and were quenched prior to analysis; no modifications to these amino acids were detected by mass spectrometry.

HOBr modified a higher percentage of the digest fragments; this may be due to differences in the size and oxidation reduction potential between HOCl and HOBr. HOBr is 30–100 times more reactive than HOCl with Trp residues and is significantly less reactive with Met residues than HOCl.\textsuperscript{43} Both hypohalous acids are more reactive than NCT.\textsuperscript{45} The susceptibility of IL-6 to HOCl exposure, in particular, is relevant to its involvement in the pathological events in COVID-19 patients. The causal role of IL-6 in a number of chronic inflammatory disorders such as rheumatoid arthritis, systemic lupus erythematosus, and Castleman’s disease is well established.\textsuperscript{48} Therapeutic interventions based on interference with IL-6 binding to IL-6R clearly provide clinical improvements.\textsuperscript{47} Most treatments are monoclonal antibodies specific for IL-6R membrane-associated proteins (e.g., Tocilizumab), though one approved for the treatment of Castleman’s disease, Siltuximab, is directed against the cytokine itself.\textsuperscript{49,50} Those same monoclonal products are now being used or are in clinical trials aimed at relief of COVID-19 signs and symptoms.\textsuperscript{9} They appear to offer significant benefits, particularly if administered at specific time points early in the onset of a disease.\textsuperscript{10,11,40} However, the administration of monoclonal antibodies is costly, requires hospitalization of patients, and adverse reactions to these biological reagents occur in an important proportion of those treated.\textsuperscript{50}

The selective chemical modifications of IL-6 that we have demonstrated after exposure to HOCl, HOBr, and NCT suggest a potential alternative approach to the mitigation of the cytokine storm and other inflammatory diseases. HOCl and NCT are known to affect events mediated by an array of cytokines, including IL-1β, IL-2, IL-4, IL-12, and IL-13.\textsuperscript{37-39,51} Our data indicate that HOCl, HOBr, and NCT are all capable of altering IL-6 binding to IL-6R.

A measure that leads to reduction of IL-6 interaction with the IL-6R in vivo could reasonably be expected to alleviate disease severity and progression. It is possible that HOCl modifications of IL-6 such as those we have described above may have, at least in part, contributed to the positive results seen when exogenous HOCl was included as part of the treatment for COVID-19 patients.\textsuperscript{52} It is also possible that NCT may be responsible for these in vivo effects since exogenous HOCl is almost instantaneously consumed in formation of NCT.\textsuperscript{33,34,40} Treatments that have the potential for self-administration avoid costly and space-limited hospitalization and warrant further studies. These are important considerations for the adoption of medical interventions in many countries where ready access to expensive resources and hospital facilities is unavailable to the great majority of the susceptible patient population.

## AUTHOR INFORMATION

### Corresponding Author

Lori I. Robins — Department of Physical Sciences, University of Washington Bothell, Bothell, Washington 98011, United States; orcid.org/0000-0003-4259-6642; Phone: +1(425)352-3208; Email: lrobins@uw.edu

### Authors

Erika K. Keim — Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, Washington 98195, United States

Deborah B. Robins — Issaquah High School, Issaquah, Washington 98027, United States

John S. Edgar — Department of Medicinal Chemistry, University of Washington, Seattle, Washington 98195, United States

John S. Meschke — Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, Washington 98195, United States

Philip R. Gafken — Fred Hutchinson Cancer Research Center, Seattle, Washington 98109, United States

Jeffrey F. Williams — Briotech Inc., Woodinville, Washington 98072, United States

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.1c05297

### Funding

This work was supported by a gift to the University of Washington Bothell School of STEM Biochemistry Research Fund from Briotech Incorporated.

### Notes

The authors declare the following competing financial interest(s): Funding: This work was supported by a gift to the University of Washington Bothell School of STEM Biochemistry Research Fund from Briotech Incorporated.
ACKNOWLEDGMENTS

We would like thank Dr. Jeremy Stone for his thoughtful review of the literature and clinical observations that brought the idea of altering inflammatory mediators to our attention.

REFERENCES

(1) Hashizume, M.; Mihara, M. The roles of interleukin-6 in the pathogenesis of rheumatoid arthritis. Arthritis 2011, 2011, 765624–765624.

(2) Tsuchida, A. I.; Beechhuizen, M.; Rutgers, M.; van Osch, G. J. V. M.; Bekkers, J. E. J.; Bot, A. G. J.; Geurts, B.; Dhert, W. J. A.; Saris, D. B. F.; Creemers, L. B. Interleukin-6 is elevated in synovial fluid of patients with focal cartilage defects and stimulates cartilage matrix production in an in vitro regeneration model. Arthritis Res. Ther. 2011, 14, R62.

(3) Henderson, L. A.; Canna, S. W.; Schulert, G. S.; Volpi, S.; Lee, P. Y.; Kernan, K. F.; Creemers, L. B. Interleukin-6 is elevated in synovial fluid of patients with focal cartilage defects and stimulates cartilage matrix production in an in vitro regeneration model. Arthritis Res. Ther. 2012, 14, 37.

(4) Hojyo, S.; Uchida, M.; Tanaka, K.; Hasebe, R.; Tanaka, Y.; Murakami, M.; Hirano, T. How COVID-19 induces cytokine storm with high mortality. Inflammation and Regeneration 2020, 40, 37–37.

(5) Herold, T.; Jurinovic, C.; Lipworth, B. J.; Hellmuth, J. C.; von Bergwelt-Baldon, M.; Klein, M.; Weinberger, T. Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. J. Allergy Clin. Immunol. 2020, 146, 128–136.e4.

(6) Conti, P.; Ronconi, G.; Caraffa, A.; Gallenga, C. E.; Ross, R.; Frydas, I.; Kritas, S. K. Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by Coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies. J. Biol. Regul. Homeostatic Agents 2020, 34, 327–331.

(7) Gabay, C. Interleukin-6 and chronic inflammation. Arthritis Res. Ther. 2006, 8, S3–S3.

(8) Patel, M.; Ikeda, S.; Pilat, S. R.; Kurzrock, R. JAK1 Genomic Alteration Associated With Exceptional Response to Silmituzumab in Cutaneous Castleman Disease. JAMA Dermatol 2017, 153, 449–452.

(9) Choy, E. H.; De Benedetti, F.; Takeuchi, T.; Hashizume, M.; John, M. R.; Kishimoto, T. Translating IL-6 biology into effective treatments. Nat. Rev. Rheumatol. 2016, 20, 335–345.

(10) Zhang, C.; Wu, Z.; Li, J.-W.; Zhao, H.; Wang, G.-Q. Cytokine release syndrome in severe COVID-19: interleukin-6 receptor antagonist tocilizumab may be the key to reduce mortality. Int. J. Antimicrob. Agents 2020, 55, 105954.

(11) Guillon, L.; Padilla, S.; fernández, M.; Aguillo, V.; García, J. A.; Telenti, G.; Garcia-Abellán, J.; Botella, A.; Gutiérrez, F.; Masiá, M. Preemptive interleukin-6 blockade in patients with COVID-19. Sci. Rep. 2020, 10, 16826.

(12) Davies, M. J.; Hawkins, C. L. The Role of Myeloperoxidase in Biomolecule Modification, Chronic Inflammation, and Disease. Antioxid. Redox Signaling 2020, 32, 957–981.

(13) Davies, M. J.; Hawkins, C. L.; Patterson, D. I.; Rees, M. D. Mammalian heme peroxides: from molecular mechanisms to health implications. Antioxid. Redox Signaling 2008, 10, 1199–1234.

(14) Winterbourn, C. C.; Kettle, A. J.; Hampton, M. B. Reactive Oxygen Species and Neutrophil Function. Annu. Rev. Biochem. 2016, 85, 765–792.

(15) Wirleitner, B.; Neurauter, G.; Nagl, M.; Fuchs, D. Down-regulatory effect of N-chlorotaurine on tryptophan degradation and neopterin production in human PBMC. Immunol. Lett. 2004, 93, 143–149.

(16) Marcinkiewicz, J.; Kontry, E. Taurine and inflammatory diseases. Amino Acids 2014, 46, 7–20.

(17) Kontry, E.; Szczepańska, K.; Kowalczyk, J.; Kurowska, M.; Janicka, I.; Marcinkiewicz, J.; Maśliński, W. The mechanism of taurine chloride inhibition of cytokine (interleukin-6, interleukin-8) production by rheumatoid arthritis fibroblast-like synoviocytes. Arthritis Rheum. 2000, 43, 2169–2177.

(18) Fukuyama, T.; Martel, B. C.; Linder, K. E.; Ehling, S.; Ganchingo, J. R.; Bäumer, W. Hypochlorous acid is antipruritic and anti-inflammatory in a mouse model of atopic dermatitis. Clinical & Experimental Allergy 2018, 48, 78–88.

(19) Fukuyama, T.; Ehling, S.; Wilczoski, J.; Bäumer, W. Comparison of topical tacitinib and 0.1% hypochlorous acid in a murine atopic dermatitis model. BMC Pharmacol. Toxicol. 2018, 19, 37.

(20) Wang, L.; Bassiri, M.; Najafi, R.; Najafi, K.; Yang, J.; Khosrov, B.; Hwong, W.; Barati, E.; Belisle, B.; Celeri, C.; Robson, M. C. Hypochlorous acid as a potential wound care agent: part II. Stabilized hypochlorous acid: a component of the inorganic armamentarium of innate immunity. J. Burns Wounds 2007, 6, No. e5.

(21) Nishimura, C.; Ekida, T.; Masuda, S.; Futatsugi, K.; Itoh, S.; Yasukawa, K.; Kishimoto, T.; Azato, Y. Chemical modification and 1H-NMR studies on the receptor-binding region of human interleukin 6. Eur. J. Biochem. 1991, 196, 377–384.

(22) Simpson, R. J.; Hammacher, A.; Smith, D. K.; Matthews, J. M.; Ward, L. D. Interleukin-6: structure-function relationships. Protein Sci. 1997, 6, 929–955.

(23) Gottardi, W.; Nagi, M. N-chlorotaurine, a natural antiseptic with outstanding tolerability. J. Antimicrob. Chemother. 2010, 65, 399–409.

(24) Gottardi, W.; Nagi, M. Chemical Properties of N-Chlorotaurine Sodium, a Key Compound in the Human Defence System. Arch. Pharm. 2002, 335, 411–421.

(25) Delgado-Enciso, I.; Paz-García, J.; Barajas-Saucedo, C. E.; Mokay-Ramírez, K. A.; Meza-Robles, C.; López-Flores, R.; Delgado-Machuca, M.; Murillo-Zamora, E.; Toscano-Velazquez, J. A.; Delgado-Enciso, J.; Melnikov, V.; Salazar-Salazar, H. R.; Delgado-Enciso, O. G.; Cabrera-Licona, A.; Guzman-Esquivel, C.; Montes-Galindo, D. A.; Hernandez-Rangel, A. E.; Montes-Díaz, P.; Rodriguez-Sanchez, I. P.; Martinez-Fierro, M. L.; Garza-Veloz, I.; Tiliburcio-Jimenez, D.; Zaiar-Fregoso, S. A.; Ramirez-Flores, M.; Gaytan-Sandoval, G.; Martinez-Perez, C. R.; Espinoza-Gómez, F.; Rojas-Larios, F.; Hirsch-Meillon, M. J.; Barrios-Navarro, E.; Oviedo-Rodriguez, V.; Rodriguez, L. M. B.; Paz-Michel, B. A. Patient-Reported Health Outcomes After Treatment of COVID-19 with Nebulized and/or Intravenous Neutral Electrolyzed Saline Combined with Usual Medical Care Versus Usual Medical care alone: A Randomized, Open-Label, Controlled Trial. Res Sq 2020, r3.srs-68403.

(26) Mainnemare, A.; Mégurbane, B.; Soueidan, A.; Daniel, A.; Chapelle, I. L. C. Hypochlorous acid and taurine-N-monochlorohyamine in periodontal diseases. J. Dent. Res. 2004, 83, 823–831.

(27) Morris, J. C. The acid ionization constant of HOCI from 5 to 35°. J. Phys. Chem. 1966, 70, 3798–3805.

(28) Kumar, K.; Margini, D. W. Kinetics and mechanism of general-acid-assisted oxidation of bromide by hypochlorite and hypochlorous acid. J. Org. Chem. 1987, 26, 2706–2711.

(29) Thomas, E. L.; Grisham, M. B.; Margaret Jefferson, M.; Preparation and characterization of chloramines. In Methods Enzymol; Academic Press: 1986; Vol. 132, pp. 569–585.

(30) Peskin, A. V.; Winterbourn, C. C. Kinetics of the reactions of hypochlorous acid and amino chloramines with thiols, methionine, and ascorbate. Free Radical Biol. Med. 2001, 30, 572–579.

(31) Hawkins, C. L.; Patterson, D. I.; Davies, M. J. Hypochlorite-induced oxidation of amino acids, peptides and proteins. Amino Acids 2003, 25, 259–274.

(32) Davies, J. M. S.; Horwitz, D. A.; Davies, K. J. A. Potential roles of hypochlorous acid and N-chloroamines in collagen breakdown by phagocytic cells in synovitis. Free Radical Biol. Med. 1993, 15, 637–643.
