Modification of IL-6 by hypochlorous acid: effects on receptor binding and possible role in treatment of COVID-19

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Abstract

Interleukin-6 (IL-6) has been implicated in the pathogenesis of acute inflammatory events in COVID-19 patients. Positive clinical responses to monoclonal antibodies directed against the IL-6 receptor (IL-6R) suggest that interference with IL-6-dependent-activation of proinflammatory pathways offers a useful approach to therapy in SARS-CoV-2 infection. We exposed IL-6 to hypochlorous acid (HOCl) *in vitro* at concentrations reported to develop *in vivo*. After HOCl treatment the cytokine failed to bind to IL-6 receptors in a bioassay using human cells engineered to provide a luminescent response to signal transduction upon receptor activation. Similar results followed exposure of IL-6 to N-chlorotaurine (NCT) and hypobromous acid (HOBr). SDS-PAGE analysis of HOCl-treated IL-6 showed neither fragmentation nor aggregation, suggesting that the modifications induced by these agents occurred on the intact protein. Mass spectrometry of intact and trypsin-digested fragments identified oxidative changes limited 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 affect the inflammatory cascade in the treatment of COVID-19. Further studies on the effects of hypohalous acids and their halogenated amine derivatives on 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

40 has now been implicated in the pathogenesis of pulmonary and systemic lesions resulting from
41 the 'cytokine storm' in seriously afflicted COVID-19 patients.[3-4] Elevated levels of IL-6 have
42 been identified as biomarkers for disease severity and are associated with hyperinflammation.[5-6]
43 Monoclonal antibodies directed against the IL-6 receptor (IL-6R) are currently used to treat
44 inflammatory diseases.[7-9] There is now compelling evidence of therapeutic benefits arising from
45 administration of these same monoclonal antibody products as part of the clinical management
46 of SARS-CoV-2 infection, particularly if the intervention is used in patients with severe disease
47 and early during treatment.[10-11] These observations make a case for IL-6 mediated events being
48 causal in the most important and often terminal consequences of COVID-19. They point up the
49 need for mitigation of such processes in the treatment and resolution of SARS-CoV-2 infections.
50 While parenteral IL-6R monoclonal antibody products may provide proven clinical benefits
51 their routine use is not realistic for this purpose in many healthcare systems around the world.
52 They are expensive, their use requires hospitalization of patients, and they can cause adverse
53 reactions that need medical attention [10,12.]

54 HOCl and other reactive oxygen species generated within phagocytic cells by the
55 myeloperoxidase system from intracellular Cl ions are known to affect the expression of
56 inflammatory cytokines, including IL-6.[12-13] Used topically, exogenous HOCl brings about
57 beneficial changes that aid in the healing and resolution of injured and infected tissues,
58 probably by at least in part modifying proteins and other substrates that contribute to the
59 pathogenesis of lesions.[14-17] HOCl readily reacts with endogenous taurine and other amines to
60 generate less reactive N-chloroamines, including N-chlorotaurine (NCT), which can also modify
61 proteins.[13]

Here we demonstrate rapid modification of IL-6 *in vitro* upon exposure to HOCl, NCT and hypobromous acid (HOBr). Markedly impaired IL-6 receptor binding was demonstrated using engineered human cells that luminesce following IL-6 induced activation of its receptor. Mass spectrometry identified changes limited to oxidation of methionine and tryptophan residues that are involved with IL-6 receptor binding.[18-19] The results suggest that hypohalous acids or NCT may have direct inhibitory effects on proinflammatory mediators such as IL-6.[20-24] They also suggest it may be worthwhile exploring this mechanism in other systemic inflammatory pathological processes in which IL-6 has been implicated.

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 (50-184-79), ELISA Stop buffer (SS03) and sodium thiosulfate ($Na_2S_2O_3$, STS) were purchased from Fisher Scientific. Sodium bromide and taurine were purchased from ACROS Organics. Water from MilliQ water purification system was used for all experiments. HOCl (pH 4) was provided by Briotech Inc. Human IL-6 was purchased from GenScript, 860 Centennial Ave. Piscataway NJ 0885 (Z03034). The IL-6 bioassay kit was purchased from Promega, 2800 Woods Hollow Road Madison, WI 53711 USA (JA2501).

Conversion of HOCl to HOBr. The conversion of HOCl to HOBr was achieved by the addition of NaBr (1.1 eq) to HOCl at pH 4. The conversion was monitored by UV-VIS (BioMate 3S). The

absorbance of HOCl at 236 nm shifted to 260 nm.[25] Test solutions were loaded in a 1 cm quartz cuvette (Sterna Cells).

Synthesis of N-chlorotaurine. The synthesis of NCT was done following literature procedures.[26-27] Briefly, HOCl was diluted with 1M NaOH to convert HOCl to $^{-}$OCl. Taurine (10 molar equivalents) was added to the solution in four aliquots with 1 min between each addition. The absorbance was measured at 250 nm. The pH was adjusted to neutral using 5 M HCl prior to use.

ELISA Assay. The Invitrogen human IL6 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 (150-200 pg/mL) was incubated with various concentrations of HOCl for 5 min and then quenched with 1% STS. The detection human IL-6 antibody conjugated with HRP was used to detect the antigen. All experiments were done a minimum of 3 times. Samples for each experiment were plated in triplicate. HOBr experiments were done following the same procedure, using fresh HOBr. NaBr (1.1 eq) was added to the stock solution of HOCl with thorough mixing for immediate use.

Trypsin Digested IL-6 Peptide Analysis by Mass Spectrometry. IL-6 and HOCl-treated IL-6 samples were prepared following the Rapid Digestion-Trypsin Protocol (Promega VA1060). . Prior to digestion, the IL-6 treated samples were prepared by incubating 25 μL of 1 mg/mL with 25 μL of HOCl (3.43 mM) and HOBr (3.43 mM). After 5 min of incubation with the hypohalous acid, the solution was quenched with 5 μL of 1% STS. The control sample was prepared by adding 25 μL of 1 mg/mL with 25 μL of water. All samples were then digested following the

provided protocol. Tandem mass spectrometry was performed for the initial HOCl-treated samples by using a Thermo Orbitrap mass spectrometer coupled to a Waters Acquity UPLC. Separations were performed on a Waters CSH 1.0 x 100 mm column. All data analysis was performed in PMI-Byonic. Additional mass spectrometry studies on HOCl- and HOBr-treated samples was carried out on a ThermoScientific Orbitrap Fusion mass spectrometer coupled to a ThermoScientific Easy nLC-1000 HPLC. Chromatographic separations were performed using a home-built 75 um x 25 cm capillary column packed with ReproSil 3 um particle size C18 material. Data were analyzed with Proteome Discoverer v2.4.

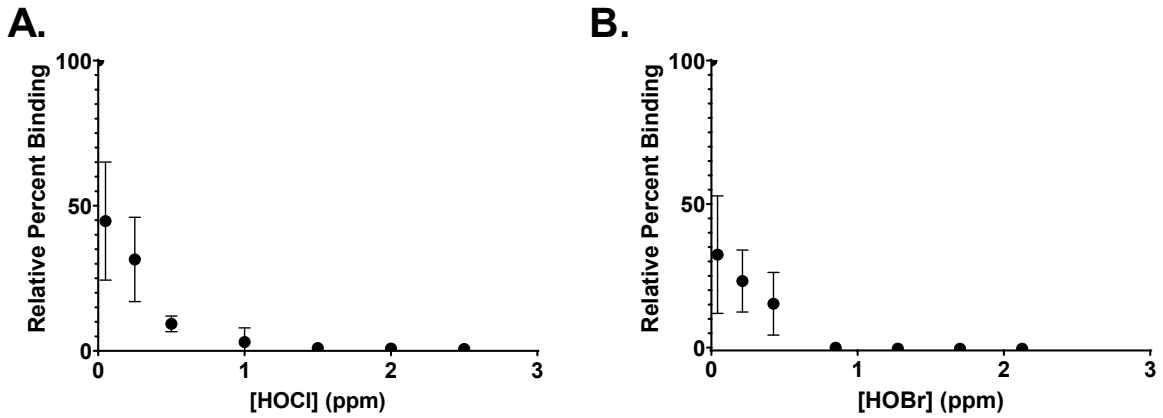
SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE). IL-6 (0.16 mg/mL) was incubated with 1.7 mM HOCl or 1.7 mM HOBr for 5 minutes. The samples were then quenched with 1 uL of 1% STS. The control sample contained IL-6 (0.16 mg/mL) in water. The samples were treated with Laemmli Sample Buffer (Biorad 1610747) 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).

IL-6 Bioassay. The IL-6 bioassay kit from Promega (JA2501) was used according to the provided protocols. HOCl-treated IL-6 was prepared by incubating 3 ug/ml IL-6 with 2.7 mM (140 ppm) and 0.27 mM (14 ppm) HOCl for 5 min prior to incubation with bioassay cells. HOBr-treated IL-6 was prepared by incubating 3ug/ml IL-6 with 1.34 mM (130 ppm) HOBr for 5 min. NCT-treated IL-6 was prepared by treating 3 ug/ml IL-6 with NCT (2.2 mM; 351 ppm) for 15 min. All HOCl, HOBr, and NCT treated IL-6 samples and IL-6 control samples were serially diluted by 3-fold in RPMI-1640 containing 10% FBS prior to application of cultured cells in IL-6

bioassay. This quenched remaining active halogen prior to the assay and was confirmed by negative tests for active chlorine (HACH 2745050). The starting concentration of IL-6 and HOCl-, HOBr- or NCT-treated HOCl was 0.33 ug/mL and was serially diluted 3-fold. The final concentrations in the presence of the cells ranged from 0.05-0.33 ug/mL. IL-6 inhibition was assayed by serially diluting 50 ug/ml anti-IL-6 receptor (ThermoFisher AHR0061) 3-fold and incubating it with the IL-6 bioassay cells 37 °C for 20 min. 12.5 ul untreated native IL-6 (1 ug/ml) was then added to the cells to test for inhibition. HOCl-treated IL-6 binding studies were accomplished by incubating serial dilutions of HOCl-, HOBr- and NCT-treated IL-6 with concentrations ranging from 1000-0.15 ng/ul for 30 min at 37 °C. 12.5 ul IL-6 (0.1 ug/ul) was then added to the cells to test for activity. 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.

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. >95% decrease in IL-6 detection was seen at HOCl concentrations greater than 1 ppm (0.02 mM). Similar results were seen with HOBr (Figure 1).



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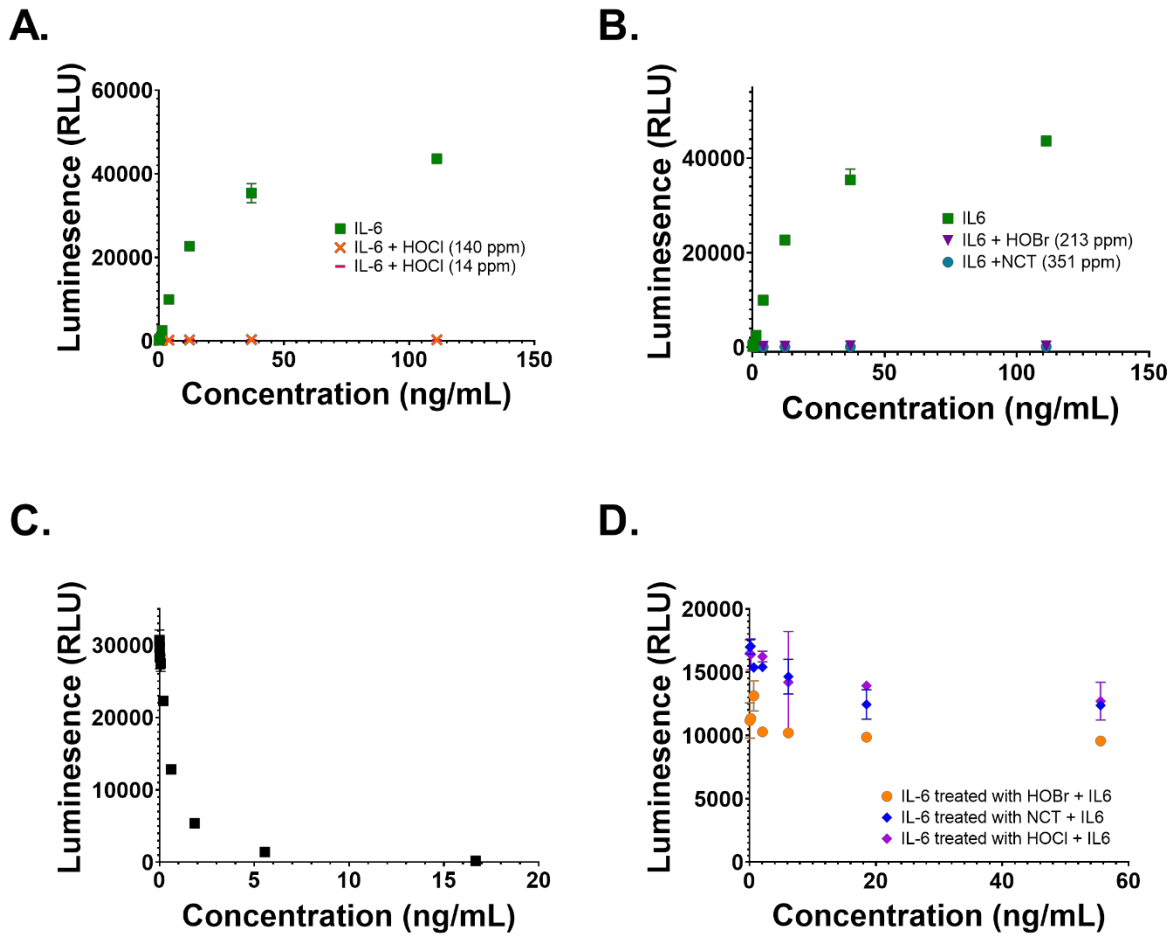
143 **Figure 1.** ELISA assay of HOCl- or HOBr-treated IL-6. A. IL-6 (150 ng/mL) was incubated with
144 various concentrations of HOCl ranging from 0-3 ppm (0-0.058 mM) for 5 min. B. IL-6 (150
145 ng/mL) was incubated with various concentrations of HOBr 0-3 ppm (0-0.031 mM) for 5 min.

146 An *in vitro* assay with human cells engineered to express the IL-6 receptor with a luciferase
147 reporter signal as an endpoint of receptor activation was used to determine if HOCl-induced
148 modifications affected the response. Unmodified IL-6 induced signal transduction measured in
149 relative luminescence units (RLU). All tested concentrations of HOCl-modified IL-6 produced
150 little to no signal transduction (Figure 2A). NCT and HOBr exposure also markedly blunted
151 signal transduction after incubation with IL-6 (Figure 2B). Binding of IL-6 to the IL-6 receptor
152 decreased in a concentration-dependent manner after the engineered cells were preincubated
153 with an anti-IL-6R antibody (Figure 2C). Modified IL-6 did not act as a receptor antagonist, as
154 demonstrated in experiments in which authentic IL-6 was added to the cells subsequent to
155 exposure to the modified protein (Figure 2D). SDS-PAGE analysis showed no fragmentation or
156 aggregation of IL-6 modified by HOCl, although concentration-dependent aggregation of IL-6
157 modified by exposure to HOBr was observed (Figure 3).



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159 **Figure 2.** *In vitro* IL-6 bioassay with engineered human cells. A. Signal transduction detection
160 by luminescence of cells incubated with various concentrations of IL-6 and IL-6 treated with
161 HOCl at 140 ppm (2.7 mM) and 14 ppm (0.27 mM). B. Signal transduction detection by
162 luminescence for cells incubated with various concentrations of IL-6 and IL-6 treated with
163 HOBr (213 ppm, 2.2 mM) and NCT (351 ppm, 2.2 mM). C. Inhibition of IL-6 binding to the IL-
164 6R in the presence of various concentrations of anti-IL-6R antibody. D. Signal transduction
165 detection for cells incubated with IL-6 pre-treated with HOCl, NCT or HOBr and then
166 subsequently treated with native IL-6.

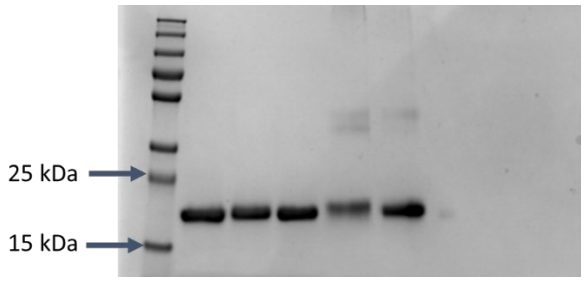

Figure 3. SDS-PAGE analysis of IL-6 treated with various concentrations of HOCl and HOBr for 5 minutes. Lane 1: Ladder; Lane 2: IL-6; Lane 3: IL-6 + HOCl (1.76 mM); Lane 4: IL-6 + HOCl (0.44 mM); Lane 5: IL-6 + HOBr (1.76 mM); and Lane 6: IL-6 + HOBr (0.44 mM).

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 the 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 presence of NCT. A single oxidation of Met 161 and dioxidation of Trp 157 was only seen in the presence of HOCl and NCT, indicating that, although the percent modification were similar to or less than the control, the products are different and not simply due to oxidations occurring during the digest. HOBr oxidation ratios were significantly higher than the control in 3/5 of the identified fragments (Figure 4C).

A. PVPPGEDSKDVAAPHRQPLTSSERIDKQIRYILDGISALRKETCNKSNMCESSKEALAENNL
NLPKMAEKDGCFQSGFNEETCLVKIITGLLEFEVYLEYLQNRFESSEEQARAVQMSTKVLI
QFLQKKAKNLDAITTPDPTTNASLLTKLQAQNQWLQDMTTHLILRSFKEFLQSSLRALRQM

B. LQAQNQWLQDMTTHLILR

C.

	Modified Peptide Fragment / Unmodified Peptide Fragment			
Modifications of Peptide Fragments	**IL-6**	**HOCl Treated IL-6**	**HOBr Treated IL-6**	**NCT Treated IL-6**
Dioxidation of Trp 157 and Met 161	0.38	0.50	0.22	0.19
Dioxidation Trp 157 or Met 161/ Oxidation of Trp 157 and Met 161	1.69	1.73	7.44	1.09
Oxidation of Met 161 and Dioxidation of Trp 157	0.00	1.64	0.00	0.97
Oxidation of Met 161 or Trp 157	3.08	2.64	6.56	1.36
Oxidation of Met 161 or Trp 157/ Dioxidation of Trp 157 or Met 161	1.69	0.00	4.22	0.00

Figure 4. Il-6 sequence and digest fragments. A. The sequence of human Il-6. B. Trypsin digest fragment containing the two modified residues shown in blue. C. Ratio of specific oxidized fragments to the unmodified fragments. Oxidized fragments that co-elute are separated with a / and combined to calculate the ratio of the modified peptide fragment to the unmodified peptide fragment.

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 rapid decline in immunological reactivity of this protein with IL-6-specific monoclonal antibodies. The inhibition of binding in the ELISA assay by HOCl- and HOBr-treated IL-6 may have resulted from only minor modifications of the cytokine such as those seen by mass spectrometry

195 analysis. SDS-PAGE analysis indicated no detectable fragmentation or aggregation of IL-6 as a
196 consequence of HOCl treatment, suggesting that alterations in antigenicity were likely brought
197 about by modifications to the intact protein.

198 Exposure to HOCl altered the ability of IL-6 to bind to the IL-6R and trigger receptor-mediated
199 generation of luminescence; signal transduction induced by treated cytokine preparations was
200 ablated at all concentrations tested. Since cells pre-incubated with HOCl-treated IL-6
201 responded normally following addition of native IL-6, we infer that HOCl inhibited the ability
202 of the treated cytokine to bind to the receptor.

203 Cytokine modifications occurred after 5 min exposure to HOCl or HOBr concentrations of
204 between 0.27 mM and 2.7 mM. These conditions are consistent with events that might occur *in*
205 *vivo*. HOCl can be produced at a rate of 134 mM/min in phagosomes and at sites of
206 inflammation, HOCl concentrations are estimated to be between 25-50 mM.[28-30]

207 HOCl has a short lifetime *in vitro* in the presence of biological molecules, estimated to be 0.1
208 μsec, which is attributable to its high reactivity and rapid modifications of substrates such as
209 sulfur containing amino acids and amines.[28, 31] Taurine, abundant in human plasma and tissues,
210 is readily chlorinated by HOCl, and NCT is well recognized as a long-lived oxidant.[32-35] HOBr,
211 in contrast, is produced *in vivo* by eosinophil granulocytes through a myeloperoxidase pathway
212 that depends upon availability of intracellular Br^- ions. The corresponding reaction product
213 with taurine is N-bromotaurine (NBT).[36] The relative molar concentrations of the two
214 hypohalous acids (HOCl and HOBr) differ markedly due to differences in Cl^- and Br^- ion
215 concentrations *in vivo*. Nonetheless, HOBr is clearly a significant factor in the reactive oxygen

species involved in host tissue responses to infection and injury; eosinophilia is associated with better clinical outcomes for COVID-19 patients.[37] HOBr and NBT, are likely contributors.

Exposure of IL-6 to freshly produced HOBr and NCT led to similar changes in reactivity in regard to receptor-mediated transmembrane responses. Signal transduction was ablated by treatment of IL-6 with these reagents, and the modified cytokine did not interfere with subsequent native IL-6 activation of the receptor. These findings suggest that comparable modifications were effected 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. 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.[18] Trp 157, a conserved residue near the receptor binding region, was modified by both HOCl and chloramine T. The structural integrity of IL-6 remained intact after treatment with chloramine T, which was also the case with HOCl.[18]

HOBr modified a higher percentage of the digest fragments; this may be due to both the size and oxidation reduction potential differences 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.[38] Both hypohalous acids are more reactive than the corresponding chlorinated taurine derivative of HOCl.[39]

The susceptibility of IL-6 to HOCl, HOBr and NCT exposure is relevant to its involvement in pathological events in COVID-19 patients. Its causal role in a number of chronic inflammatory disorders such as rheumatoid arthritis, systemic lupus erythematosus and Castleman's disease

237 is well established.[7-8] Therapeutic interventions in these diseases based upon interference with
238 IL-6 binding to IL-6R clearly provide clinical improvements.[40] Most treatments are monoclonal
239 antibodies specific for IL-6R membrane-associated proteins (e.g., Tocilizumab), though one
240 approved for treatment of Castleman's disease, Siltuximab, is directed against the cytokine
241 itself.[7-8, 41] Those same monoclonal products are now being used or are in clinical trials aimed at
242 relief of COVID-19 signs and symptoms.[9] They appear to offer significant benefits, particularly
243 if administered at specific time points early in the onset of disease.[10-11, 42] The administration of
244 monoclonal antibodies is costly and requires hospitalization of patients, and adverse reactions
245 to these biological reagents occur in an important proportion of those treated.[43]

246 The selective chemical modifications of IL-6 that we have demonstrated after exposure to HOCl,
247 HOBr and NCT suggest an alternative approach to mitigation of the cytokine storm. HOCl is
248 widely used topically in clinical management of many infectious and inflammatory conditions,
249 and both the hypohalous acid and NCT are known to affect events mediated by an array of
250 cytokines, including IL-1B, IL-2, Il-4, IL-12 and IL-13.[14-16, 44] Our data indicate that HOCl, HOBr
251 and NCT are all capable of modifying IL-6 with comparable effects on IL-6R binding. The
252 differences in the reactivity of these oxidants with specific amino acid residues may lead to
253 important differential effects of these natural products on the diversity of biologically active
254 polypeptides that participate in proinflammatory processes.[29, 39, 45-47] However, HOCl is almost
255 instantaneously consumed in the conversion of taurine into NCT so the likelihood is high that
256 any beneficial effects of exogenous HOCl *in vivo* are due to generation of NCT.[21, 27]

Future studies may open up the potential for *in vivo* use of HOCl formulations for viral infections and inflammatory related diseases, including those caused by SARS-CoV-2. IL-6 is typically present at elevated levels in COVID-19 patients both systemically and locally in pulmonary tissues and exudates.[48] 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. The advantages of exposure to formulations of these compounds via inhalation, in particular, arise from the potential for self-administration and avoidance of costly hospitalization. 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.

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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.; Beekhuizen, 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. *Arthrit. Res. Ther.* **2012,** *14* (6), R262.
3. Henderson, L. A.; Canna, S. W.; Schulert, G. S.; Volpi, S.; Lee, P. Y.; Kernan, K. F.; Caricchio, R.; Mahmud, S.; Hazen, M. M.; Halyabar, O.; Hoyt, K. J.; Han, J.; Grom, A. A.; Gattorno, M.; Ravelli, A.; De

Benedetti, F.; Behrens, E. M.; Cron, R. Q.; Nigrovic, P. A., On the Alert for Cytokine Storm: Immunopathology in COVID-19. *Arthritis & rheumatology (Hoboken, N.J.)* **2020,** *72* (7), 1059-1063.
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* (1), 37-37.
5. Herold, T.; Jurinovic, V.; Arnreich, C.; Lipworth, B. J.; Hellmuth, J. C.; von Bergwelt-Baildon, 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* (1), 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. *Journal of biological regulators and homeostatic agents* **2020,** *34* (2), 327-331.
7. Gabay, C., Interleukin-6 and chronic inflammation. *Arthrit. Res. Ther.* **2006,** *8 Suppl 2* (Suppl 2), S3-S3.
8. Patel, M.; Ikeda, S.; Pilat, S. R.; Kurzrock, R., JAK1 Genomic Alteration Associated With Exceptional Response to Siltuximab in Cutaneous Castleman Disease. *JAMA Dermatol* **2017,** *153* (5), 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. *Nature Reviews Rheumatology* **2020,** *16* (6), 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* (5), 105954.
11. Guillén, L.; Padilla, S.; Fernández, M.; Agulló, V.; García, J. A.; Telenti, G.; García-Abellán, J.; Botella, Á.; Gutiérrez, F.; Masiá, M., Preemptive interleukin-6 blockade in patients with COVID-19. *Scientific Reports* **2020,** *10* (1), 16826.
12. 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* (2), 143-149.
13. Winterbourn, C. C.; Kettle, A. J.; Hampton, M. B., Reactive Oxygen Species and Neutrophil Function. *Annu. Rev. Biochem* **2016,** *85*, 765-92.
14. Fukuyama, T.; Martel, B. C.; Linder, K. E.; Ehling, S.; Ganchingco, J. R.; Bäumer, W., Hypochlorous acid is antipruritic and anti-inflammatory in a mouse model of atopic dermatitis. *Clinical & Experimental Allergy* **2018,** *48* (1), 78-88.
15. Fukuyama, T.; Ehling, S.; Wilzopolski, J.; Bäumer, W., Comparison of topical tofacitinib and 0.1% hypochlorous acid in a murine atopic dermatitis model. *BMC Pharmacology and Toxicology* **2018,** *19* (1), 37.
16. Kontny, E.; Szczepańska, K.; Kowalczewski, J.; Kurowska, M.; Janicka, I.; Marcinkiewicz, J.; Maśliński, W., The mechanism of taurine chloramine inhibition of cytokine (interleukin-6, interleukin-8) production by rheumatoid arthritis fibroblast-like synoviocytes. *Arthritis Rheum.* **2000,** *43* (10), 2169-77.
17. Wang, L.; Bassiri, M.; Najafi, R.; Najafi, K.; Yang, J.; Khosrovi, B.; Hwong, W.; Barati, E.; Belisle, B.; Celeri, C.; Robson, M. C., Hypochlorous acid as a potential wound care agent: part I. Stabilized hypochlorous acid: a component of the inorganic armamentarium of innate immunity. *Journal of burns and wounds* **2007,** *6*, e5.
18. Nishimura, C.; Ekida, T.; Masuda, S.; Futatsugi, K.; Itoh, S.; Yasukawa, K.; Kishimoto, T.; Arata, Y., Chemical modification and 1H-NMR studies on the receptor-binding region of human interleukin 6. *Eur. J. Biochem.* **1991,** *196* (2), 377-84.
19. Simpson, R. J.; Hammacher, A.; Smith, D. K.; Matthews, J. M.; Ward, L. D., Interleukin-6: structure-function relationships. *Protein Sci.* **1997,** *6* (5), 929-55.

20. Michaela, L.; Annika, R.; André, V.; Marlena, S.; Brigitte, M.; Zoltan, B.; Johannes, S.; Angela, L.; Jennifer, F.; Heribert, S.; Dorothee von, L.; Thorsten, W.; Carsten, S.; Markus, N., *Res Sq* **2021**.
21. Gottardi, W.; Nagl, M., N-chlorotaurine, a natural antiseptic with outstanding tolerability. *J. Antimicrob. Chemother.* **2010,** *65* (3), 399-409.
22. Gottardi, W.; Nagl, M., Chemical Properties of N-Chlorotaurine Sodium, a Key Compound in the Human Defence System. *Arch. Pharm.* **2002,** *335* (9), 411-421.
23. Delgado-Enciso, I.; Paz-Garcia, J.; Barajas-Saucedo, C. E.; Mokay-Ramírez, K. A.; Meza-Robles, C.; Lopez-Flores, R.; Delgado-Machuca, M.; Murillo-Zamora, E.; Toscano-Velazquez, J. A.; Delgado-Enciso, J.; Melnikov, V.; Walle-Guillen, M.; Galvan-Salazar, H. R.; Delgado-Enciso, O. G.; Cabrera-Licona, A.; Guzman-Esqu, J.; Montes-Galindo, D. A.; Hernandez-Rangel, A. E.; Montes-Diaz, P.; Rodriguez-Sanchez, I. P.; Martinez-Fierro, M. L.; Garza-Veloz, I.; Tiburcio-Jimenez, D.; Zaizar-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**, rs.3.rs-68403.
24. Mainnemare, A.; Mégarbane, B.; Soueidan, A.; Daniel, A.; Chapple, I. L., Hypochlorous acid and taurine-N-monochloramine in periodontal diseases. *J. Dent. Res.* **2004,** *83* (11), 823-31.
25. Kumar, K.; Margerum, D. W., Kinetics and mechanism of general-acid-assisted oxidation of bromide by hypochlorite and hypochlorous acid. *Inorg. Chem.* **1987,** *26* (16), 2706-2711.
26. Thomas, E. L.; Grisham, M. B.; Margaret Jefferson, M., [40] Preparation and characterization of chloramines. In *Methods Enzymol.*, Academic Press: 1986; Vol. 132, pp 569-585.
27. Peskin, A. V.; Winterbourn, C. C., Kinetics of the reactions of hypochlorous acid and amino acid chloramines with thiols, methionine, and ascorbate. *Free Radic Biol Med* **2001,** *30* (5), 572-579.
28. Winterbourn, C. C.; Hampton, M. B.; Livesey, J. H.; Kettle, A. J., Modeling the reactions of superoxide and myeloperoxidase in the neutrophil phagosome: implications for microbial killing. *J. Biol. Chem.* **2006,** *281* (52), 39860-9.
29. Summers, F. A.; Morgan, P. E.; Davies, M. J.; Hawkins, C. L., Identification of Plasma Proteins That Are Susceptible to Thiol Oxidation by Hypochlorous Acid and N-Chloramines. *Chem. Res. Toxicol.* **2008,** *21* (9), 1832-1840.
30. Weiss, S. J., Tissue Destruction by Neutrophils. *New Engl. J. Med.* **1989,** *320* (6), 365-376.
31. Schürmann, N.; Forrer, P.; Casse, O.; Li, J.; Felmy, B.; Burgener, A.-V.; Ehrenfeuchter, N.; Hardt, W.-D.; Recher, M.; Hess, C.; Tschan-Plessl, A.; Khanna, N.; Bumann, D., Myeloperoxidase targets oxidative host attacks to Salmonella and prevents collateral tissue damage. *Nature Microbiology* **2017,** *2* (4), 16268.
32. Marcinkiewicz, J.; Nowak, B.; Grabowska, A.; Bobek, M.; Petrovska, L.; Chain, B., Regulation of murine dendritic cell functions in vitro by taurine chloramine, a major product of the neutrophil myeloperoxidase–halide system. *Immunology* **1999,** *98* (3), 371-378.
33. Wu, G., Important roles of dietary taurine, creatine, carnosine, anserine and 4-hydroxyproline in human nutrition and health. *Amino Acids* **2020,** *52* (3), 329-360.
34. Weiss, S. J.; Lampert, M. B.; Test, S. T., Long-lived oxidants generated by human neutrophils: characterization and bioactivity. *Science (New York, N.Y.)* **1983,** *222* (4624), 625-8.
35. Grisham, M. B.; Jefferson, M. M.; Melton, D. F.; Thomas, E. L., Chlorination of endogenous amines by isolated neutrophils. Ammonia-dependent bactericidal, cytotoxic, and cytolytic activities of the chloramines. *J. Biol. Chem.* **1984,** *259* (16), 10404-13.
36. Marcinkiewicz, J.; Mak, M.; Bobek, M.; Biedroń, R.; Białecka, A.; Koprowski, M.; Kontny, E.; Maśliński, W., Is there a role of taurine bromamine in inflammation? Interactive effects with nitrite and

hydrogen peroxide. *Inflammation research : official journal of the European Histamine Research Society ... [et al.]* **2005,** *54* (1), 42-9.
37. Tanni, F.; Akker, E.; Zaman, M. M.; Figueroa, N.; Tharian, B.; Hupart, K. H., Eosinopenia and COVID-19. *The Journal of the American Osteopathic Association* **2020,** *120* (8), 504-508.
38. Pattison, D. I.; Davies, M. J., Kinetic analysis of the reactions of hypobromous acid with protein components: implications for cellular damage and use of 3-bromotyrosine as a marker of oxidative stress. *Biochemistry* **2004,** *43* (16), 4799.
39. De Carvalho Bertozo, L.; Morgon, N. H.; De Souza, A. R.; Ximenes, V. F., Taurine Bromamine: Reactivity of an Endogenous and Exogenous Anti-Inflammatory and Antimicrobial Amino Acid Derivative. *Biomolecules* **2016,** *6* (2), 23.
40. Ireland, S. J.; Monson, N. L.; Davis, L. S., Seeking balance: Potentiation and inhibition of multiple sclerosis autoimmune responses by IL-6 and IL-10. *Cytokine* **2015,** *73* (2), 236-44.
41. Sarosiek, S.; Shah, R.; Munshi, N. C., Review of siltuximab in the treatment of multicentric Castleman's disease. *Ther Adv Hematol* **2016,** *7* (6), 360-366.
42. Lisi, L.; Lacal, P. M.; Barbaccia, M. L.; Graziani, G., Approaching coronavirus disease 2019: Mechanisms of action of repurposed drugs with potential activity against SARS-CoV-2. *Biochem. Pharmacol.* **2020,** *180*, 114169-114169.
43. Hansel, T. T.; Kropshofer, H.; Singer, T.; Mitchell, J. A.; George, A. J., The safety and side effects of monoclonal antibodies. *Nat. Rev. Drug Discov.* **2010,** *9* (4), 325-38.
44. Pelgrift, R. Y.; Friedman, A. J., Topical Hypochlorous Acid (HOCl) as a Potential Treatment of Pruritus. *Current Dermatology Reports* **2013,** *2* (3), 181-190.
45. Vissers, M. C.; Carr, A. C.; Chapman, A. L., Comparison of human red cell lysis by hypochlorous and hypobromous acids: insights into the mechanism of lysis. *The Biochemical journal* **1998,** *330 (Pt 1)* (Pt 1), 131-138.
46. Hawkins, C. L.; Davies, M. J., Inactivation of protease inhibitors and lysozyme by hypochlorous acid: role of side-chain oxidation and protein unfolding in loss of biological function. *Chem. Res. Toxicol.* **2005,** *18* (10), 1600-10.
47. Ximenes, V. F.; Morgon, N. H.; de Souza, A. R., Hypobromous acid, a powerful endogenous electrophile: Experimental and theoretical studies. *J. Inorg. Biochem.* **2015,** *146*, 61-68.
48. Abers, M. S.; Delmonte, O. M.; Ricotta, E. E.; Fintzi, J.; Fink, D. L.; de Jesus, A. A. A.; Zarember, K. A.; Alehashemi, S.; Oikonomou, V.; Desai, J. V.; Canna, S. W.; Shakoory, B.; Dobbs, K.; Imberti, L.; Sottini, A.; Quiros-Roldan, E.; Castelli, F.; Rossi, C.; Brugnoni, D.; Biondi, A.; Bettini, L. R.; D'Angio, M.; Bonfanti, P.; Castagnoli, R.; Montagna, D.; Licari, A.; Marseglia, G. L.; Gliniewicz, E. F.; Shaw, E.; Kahle, D. E.; Rastegar, A. T.; Stack, M.; Myint-Hpu, K.; Levinson, S. L.; DiNubile, M. J.; Chertow, D. W.; Burbelo, P. D.; Cohen, J. I.; Calvo, K. R.; Tsang, J. S.; Consortium, N. C.-.; Su, H. C.; Gallin, J. I.; Kuhns, D. B.; Goldbach-Mansky, R.; Lionakis, M. S.; Notarangelo, L. D., An immune-based biomarker signature is associated with mortality in COVID-19 patients. *JCI Insight* **2021,** *6* (1), e144455.