Excess Iodine Exposure Acutely Increases Salivary Iodide And Antimicrobial Hypoiodous Acid Concentrations In Humans

Yasutada Akiba
Gastroenterology and Hepatology
Angela M. Leung
Endocrinology, Diabetes, and Metabolism
Muhammad-Tariq Bashir
Greater Los Angeles VA Healthcare System
Ramin Ebrahimi
David Geffen School of Medicine at UCLA
Jesse W. Currier
David Geffen School of Medicine at UCLA
Natalia Neverova
David Geffen School of Medicine at UCLA
Jonathan D Kaunitz (jake@ucla.edu)
Gastroenterology and Hepatology

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Abstract

The lactoperoxidase (LPO)-hydrogen peroxide-halides reaction (LPO system) converts iodide and thiocyanate (SCN^-) into hypoiodous acid (HOI) and hypothiocyanite ($OSCN^-$), respectively. Since this system has been implicated in defense of the airways and oropharynx from microbial invasion, we measured the concentrations of these analytes in human saliva before and after iodine administration to test the hypothesis that an iodide load increases salivary iodide and HOI concentrations. Salivary iodide, SCN^-, HOI and $OSCN^-$ were measured using standard methodology. Salivary iodide and HOI levels significantly increased after iodinated contrast injection compared with baseline levels, whereas there was no significant change in salivary SCN^- and $OSCN^-$ levels. The contrast dye iodine load and changes of salivary iodide and HOI levels were positively correlated, suggesting that higher iodide in the circulation increases iodide output and salivary HOI production. Excess iodine exposure in humans increases the salivary output of iodide, increasing salivary HOI concentrations with no effect on $SCN^-/OSCN^-$ levels. This first of its kind study suggests that a sufficient but safe iodide supplementation may augment the generation of antimicrobial HOI by the salivary LPO system against airborne viral pathogens, including coronaviruses and influenza viruses, a possible inexpensive means of effectively curbing viral pandemics.

Introduction

Salivary lactoperoxidase (LPO) catalyzes the oxidation of the halide iodide and pseudohalide thiocyanate (SCN^-) to the strongly oxidant disinfectant hypohalites hypoiodous acid (HOI) and hypothiocyanite ($OSCN^-$), respectively, in the presence of hydrogen peroxide (H_2O_2). This reaction, termed the LPO system, contributes to the important anti-bacterial and anti-viral protective host defense mechanisms at the oral, respiratory, and gastrointestinal epithelial interfacial surfaces [1–3]. The LPO system is regarded as a first line of defense for airborne and aerosolized infections by bacteria and viruses. Products generated by the LPO system (iodide/HOI or $SCN^-/OSCN^-$) have been suggested to have antiviral activity for enveloped viruses such as respiratory syncytial virus [4,5], influenza viruses [6,7], and herpes simplex virus type 1 [8]. Generation of hypohalites by the LPO system is akin to the generation of hypochlorous acid (HClO) by myeloperoxidase in neutrophils with the exception that LPO has low affinity to chloride ion that cannot generate HClO. Furthermore, in contrast to the NADPH oxidase (Nox) in neutrophils [9], salivary, respiratory and gastrointestinal luminal H_2O_2 is supplied by other members of Nox family, termed dual oxidases (Duox): airway epithelium predominantly expresses Duox1 [2], whereas salivary ductal and intestinal epithelia express Duox2 [10,11]. Iodide and SCN^-, the primary LPO substrates, are taken up from the bloodstream via the sodium iodide symporter (NIS; SLC5A5) at the basolateral membrane of salivary duct cells and gastric epithelial cells as well as the thyroid follicles [12], followed by the secretion into the saliva or gastric lumen through the anion channels cystic fibrosis transmembrane conductance regulator (CFTR) or anoctamin-1 (ANO1, also known as transmembrane member 16A; TMEM16A), or the anion exchanger pendrin (SLC26A4) at the apical membrane [13].

The current COVID-19 pandemic has severely impacted the health and wellbeing of humanity, with over 268 million cases and 5.3 million deaths reported globally as of December 9, 2021 [14]. Although vaccinations have effectively curbed the propagation of the current viral subtypes, there are no proven medical treatments available for infection with the enveloped virus SARS-CoV-2, with the bulk of efforts devoted to reducing the spread of the disease through traditional epidemiologic-guided measures such as social distancing, handwashing, and mask wearing. The infection rate of SARS-CoV-2 and COVID-19 mortality rates vary widely among countries. One apparent anomaly is the low per capita infection rate among Asian countries despite their high urban population density. For example, although the case fatality rates among the US, Japan, and South Korea are roughly comparable at 1.6%, 1.1%, and 0.8%, respectively, the deaths/100k population are quite different at 242, 14.6, and 7.9 as of December 9, 2021 [14], reflecting much lower per capita infection (and presumably transmission) rates in these Asian countries despite their high population densities and urban crowding with consequent lack of social distancing in public conveyances such as buses, trains and elevators. Although many factors contribute to these statistics such as the widespread use of masks, the effectiveness of quarantines, and efficient public health systems, another seldom-discussed factor is the iodine content in the diet. In particular, since marine plants such as kelp and seaweed concentrate iodine from seawater up to 150,000 fold [15], their consumption can increase dietary iodine consumption from the mean amount of 150 µg/day in the US [16,17] to 1-2 mg/day or more in Japan [18,19]. Detailed studies have reported that salivary iodine concentrations and secretion rates are highly correlated with dietary iodine consumption [20]. Thus, increasing daily iodine intake from 150 µg/day to the US Institute of Medicine upper limit of intake of 1,100 µg/day [16] should increase salivary iodine (and presumably HOI) concentrations over 7-fold. We hypothesize that the upper limit of iodine consumption in Asian diets is adequate to produce viral suppressive HOI concentrations in the saliva.

Despite the promise of this endogenous antiviral system towards reducing the transmission of respiratory viruses, analyte levels of the salivary LPO system have not been studied in humans. We hypothesize that an excess iodide load to the bloodstream may increase salivary iodide secretion and result in an enhanced salivary LPO system in humans. Thus, we examined the salivary levels of iodide, SCN^-, HOI and $OSCN^-$ after iodinated contrast administration routinely used in patients undergoing coronary angiography.

Materials And Methods

Human Subjects and Saliva Sample Collections

Inclusion criterion: Subjects who had been scheduled to undergo coronary angiography as part of usual care at the West Los Angeles Veterans Affairs Medical Center were enrolled into the study. Exclusion criteria: 1) refusal to be included in the study; 2) inability to provide informed consent; and 3) inability or refusal to provide an adequate saliva sample. Data analysis was performed on only the subset of

subjects who provided the baseline saliva sample and at least one post-iodinated contrast exposure salivary specimen of sufficient volume for the laboratory measurements.

There was a total of 49 subjects prospectively enrolled from August 20, 2020 to September 9, 2021. All subjects had a negative COVID-19 PCR test confirmed before coronary angiography. Subjects underwent catheterization for coronary angiography during which 25-230 ml of iodinated contrast medium (Visipaque®-320) was injected intraarterially so as to visualize the coronary arteries. In study 1 (n = 30), saliva was collected at two timepoints: immediately before and 3-6 hours after iodinated contrast exposure; 6 subjects were excluded due to insufficient saliva volume. In study 2 (n = 19), saliva was collected at up to 5 timepoints: immediately before and 4 additional times up to 48 hours after iodinated contrast exposure; 3 subjects were excluded due to insufficient saliva or cancellation of the coronary angiography procedure. Therefore, data for 24 subjects for study 1 and 16 subjects for study 2 were analyzed.

Saliva was collected using a universal saliva collection kit (Super SAL, Oasis Diagnosis, Vancouver, WA, USA). Saliva specimens were stored at 4˚C and centrifuged at 1,000xg for 5 min on the day of each collection. The supernatant was stored at -20˚C until analysis. The study was performed with approval of the Greater Los Angeles Veterans Affairs Healthcare System Institutional Review Board (IRB) and is compliant with all institutional policies covering human research. Written informed consent was obtained from each enrolled subject. This research conformed to the standards set by the latest version of the *Declaration of Helsinki* or the version that was in place at the time of the experiments. Note that since the sole intervention used for the research was the collection of saliva, this study was considered to be “minimal risk”. Under the study terms dictated by the IRB, no patient-level descriptive data such as age, sex, gender, diagnosis, etc. were recorded.

Chemicals

Ionic strength adjustors (ISA) solution for iodide was purchased from Thermo Fisher Scientific (#940011, Waltham, MA, USA). Sodium iodide (NaI), iodine, sodium thiocyanate (NaSCN), lactoperoxidase (LPO), 5,5’-dithiobis-(2-nitrobenzoic acid) (DTNB) and β-nicotinamide adenine dinucleotide 2’-phosphate reduced tetrasodium salt hydrate (β-NADPH), ferric chloride ($FeCl_3$), hydrogen peroxide (H_2O_2) and other chemicals were purchased from Sigma Chemical (St. Louis, MO, USA). Iodine was freshly dissolved in 95% ethanol. All other solutions were made with Milli-Q water.

Laboratory Measurements

Iodide

The iodide content in the saliva was measured using an Orion iodide electrode (Thermo Fisher Scientific). The iodide selective electrode was selective for iodide in the range of 1 μM – 1 mM over SCN^- or Cl^-. A standard curve for NaI (1 μM – 1 mM) in 10% ISA solution was made every time. Fifty μl of the saliva sample was added to 450 μl of 10% ISA solution and then mV was read after stabilization.

SCN^-

The SCN^- content in the saliva was measured by ferric colorization. Twenty-five µl of NaSCN standard (10 µM – 2.5 mM) in Tris buffer (50 mM, pH 7.4) or samples was placed in a clear flat-bottom 384-well plate (Nunc, Thermo Fisher Scientific) and 25 µl of $FeCl_3$ (0.1 M) in Tris buffer was added into each well. The absorbance at 490 nm was read using a multi-mode microplate reader (Synergy-2, BioTek Instruments, Inc., Winooski, VT, USA). The yellow color of $FeCl_3$ will be immediately turns to deep red according to the production of $[Fe(SCN)]^{2+}$. Grossly reddish saliva samples with high background absorbance at 490 nm were omitted from the analysis due the high likelihood of inaccurate measurements.

$OSCN^-$

The $OSCN^-$ content in the saliva was measured using Ellman's reagent according to the previous report [21]. DTNB 10 mM 6.4 µl was added to Tris buffer (992.6 µl, 50 mM, pH 7.4), followed by the addition of 1-µl of 2-mercaptoethanol (60 mM) to make yellow TNB solution (60 µM). SCN^- standard (2 µM – 2 mM) was made from 1M NaSCN stock solution in Tris buffer. LPO 100 µg/ml 40 µl and H_2O_2 10 mM 8 µl were added in Tris buffer total 1 ml to make the reaction solution containing LPO 4 µg/ml and H_2O_2 80 µM. Equal volume of SCN^- standard and the reaction solution was mixed to generate $OSCN^-$ standard solution. Twenty-five µl of $OSCN^-$ standard or samples was placed in a clear flat-bottom 384-well plate and 25-µl of TNB solution freshly prepared was added to each well. The absorbance at 412 nm was read for 10 min with 2-min interval using Synergy-2. The yellow color of TNB solution was diminished by the oxidation of TNB to DTNB (colorless) by the presence of $OSCN^-$ in the standard and samples. Background absorbance of samples at 412 nm was also measured separately and subtracted from the $OSCN^-$ measured values.

HOI

The HOI content in the saliva was measured using NADPH conversion to iodinated NADPH (NADPI) according to the previous report [22]. *In situ* production of HOI from NaI with LPO and H_2O_2 was possible but with a lower conversion rate. NADPI was freshly prepared as previously reported [23] and used as chemical standard. Ten-µl of iodine solution (10 mM in 95% ethanol) and 1 µl of NADPH (0.1 M) were added to Tris buffer to make 1 ml of 100 µM NADPI solution. The absorbance of NADPH at 340 nm was rapidly bleached by the addition of iodine, with complete conversion of NADPH to NADPI. The samples (25 µl) were reacted with 25 µl of 500 µM NADPH in Tris buffer in a clear flat-bottom 384-well plate. The absorbances of blank (Tris buffer only), NADPI standards and the reacted samples at 340 nm for NADPH and 282 nm for NADPI were read for 20 min at 2-min intervals using the Synergy-2 instrument. According to HOI content, the 340 nm reading (NADPH) was reduced, whereas the 282 nm reading (NADPI) was increased. Background absorbance of samples at 340 and 282 nm was also measured separately and

subtracted from the HOI measured values. Increased absorbance at 282 nm was used to calculate the NADPI content, equivalent to the HOI content in the samples.

Statistical Analysis

Values are individually plotted as before and after pairs and expressed as median [interquartile range (IQR)]. Statistical analysis was performed with GraphPad® Prism 9 (La Jolla, CA, USA) using Wilcoxon matched-pairs signed rank test, two-tailed, or Friedman test, two-tailed, followed by Dunn's multiple comparison test. Correlation between iodide content in the injected contrast medium and the changes in iodide levels in saliva, or between the levels of iodide and HOI, was assessed by simple linear regression and Spearman correlation. Differences were considered significant when *P* values were < 0.05.

Results

Salivary contents of iodide, SCN^-, HOI and $OSCN^-$ before and after iodinated contrast exposure. In study 1, salivary iodide concentrations (μM) were increased after iodinated contrast exposure (median [IQR]; pre 16.7 [9.6-41.9], 3-6 hr 331.9 [145.5-654.7], p < 0.0001) (Fig. 1A). In parallel, salivary HOI concentrations (μM) were increased after iodinated contrast exposure (pre 1.6 [0.9-2.3], 3-6 hr 5.1 [3.6-7.1], p < 0.0001) (Fig. 1C). Conversely, there were no changes in salivary levels of SCN^- (μM) and $OSCN^-$ (μM) before and after iodinated contrast exposure; SCN^-, pre 1471 [815.1-2575], 3-6 hr 1633 [693.3-2661], p = 0.7469; $OSCN^-$, pre 8.0 [3.0-11.8], 3-6 hr 8.1 [3.2-17.5], p = 0.1974 (Fig. 1B, 1D).

In study 2, salivary iodide concentrations were increased over time following iodinated contrast exposure (0 hr 24.3 [19.6-36.0], 6 hr 161.1 [102.0-283.4], 24 hr 256.6 [70.6-440.0]) (Fig. 2A). Salivary levels of HOI after iodinated contrast exposure was also higher than the basal level at 0 hr (0 hr 3.2 [1.7-7.4], 6 hr 8.6 [3.1-11.8], 24 hr 5.8 [3.5-12.4]) (Fig. 2C). In contrast, there were no changes in salivary levels of SCN^- and $OSCN^-$ before and after iodinated contrast exposure; SCN^-, 0 hr 1854 [1010-3117], 6 hr 1281 [905.0-1883], 24 hr 1081 [717.1-1715]; $OSCN^-$, 0 hr 5.0 [3.6-8.6], 6 hr 9.9 [3.1-17.0], 24 hr 4.7 [3.1-11.9] (Fig. 2B, 2D). In 4 subjects, saliva was collected up to 48 hr; salivary iodide levels at 48 hr were higher than the basal levels, but not significant (0 hr 19.9 [16.9-20.9], 48 hr 68.1 [43.5-861.7], p = 0.125).

Correlations between iodinated contrast load, salivary levels of iodide, and salivary HOI levels

We analyzed the correlations between the iodine load (g), as calculated from the iodine content (320 mg/ml) and volume used (ml) of the contrast medium, and the changes in salivary iodide levels (μM) before and after iodinated contrast exposure from both studies (n = 39 pairs; one missing this information). The changes in salivary iodide levels (Δiodide) were positively correlated with the iodine load (simple linear regression; R^2 = 0.2369, p = 0.0017, Spearman correlation; r = 0.4868, p = 0.0017) (Fig. 3A), suggesting that higher free iodide levels in the circulation increases salivary iodide output.

We also plotted salivary levels of iodide versus HOI of all data from both studies (n = 96 pairs). HOI levels were positively correlated with iodide levels (simple linear regression; $R^2 = 0.1536$, $p < 0.0001$, Spearman correlation; $r = 0.4729$, $p < 0.0001$) (Fig. 3B), suggesting that the increased iodide output in saliva enhances the production of HOI in saliva.

Discussion

In this first report of the measurement of the antimicrobial hypohalite HOI in human saliva, we report the effects of an excess iodide load on the salivary output of halide iodide, pseudo-halide SCN^-, and the corresponding antimicrobial oxidants HOI and $OSCN^-$ in humans. Iodine is a micronutrient needed for the synthesis of thyroid hormone [24]. A single dose of iodinated contrast medium supplies several hundredfold the daily recommended requirement of iodine [25]. We demonstrated that iodinated contrast exposure proportionally increases salivary iodide output with a concomitant increase of HOI output in saliva. This is also the first study demonstrating that a supraphysiologic iodine load acutely increased salivary iodide output and that HOI content in saliva was well correlated with increased iodide output in humans. These results strongly suggest that an excess iodide load acutely increases salivary iodide output and enhances antimicrobial HOI production in saliva.

The SARS-CoV-2 virus infects and replicates in a subpopulation of oral cells and salivary gland cells that express the ectoenzymes angiotensin converting enzyme 2 (ACE2) and transmembrane protease, serine 2 (TMPRSS2) [26]. Saliva from asymptomatic patients with COVID-19 contains infectious virus [26], suggesting that saliva is the source of airborne infection of SARS-CoV-2, and that saliva is a potential therapeutic target for the treatment and prevention of COVID-19. Although intermittent oral rinses or mouthwash with antimicrobial solutions containing ethanol, hydrogen peroxide and povidone-iodine have been proposed for preventing transmission of SARS-CoV-2 [27,28], the effects of these interventions are transient, thus requiring a sustainable supply of antimicrobial compounds in order to prevent transmission of and infection with SARS-CoV-2. Enhanced and sustained output of endogenously generated antimicrobial oxidant species, such as HOI and $OSCN^-$, would be better suited for this purpose.

To date, the COVID-19 pandemic has killed 5.3 million people worldwide [14]. Although the rapid development of effective mRNA-based vaccines is a triumph of modern medicine, the high mutation rate of SARS-CoV-2 increases the probability that a vaccine-resistant strain may eventually emerge. Furthermore, the high cost of manufacturing and delivering vaccines has impaired inoculation of citizens of nations in the developing world, with consequent overtaxing of medical resources, negative economic impact, and high mortality. Clearly, an inexpensive means to slow the transmission of enveloped respiratory viruses would have enormous impact on the current and predicted future viral pandemics. Based on these preliminary human data, we support the proposal by Smith et al [29] that the provision of iodine supplements in nontoxic doses to at-risk populations, by impeding viral transmission, may inexpensively curb pandemics due to respiratory enveloped viruses, an hypothesis that can tested with larger scale clinical trials.

The antimicrobial effects of hypohalous acids such as HOI on enveloped viruses is variable and unpredictable. Although the few in vivo experiments of iodine supplementation in sheep or cattle infected with respiratory viruses have yielded promising results, the pathogenic microorganisms tested did not include coronaviruses [5,30,31]. Patel et al measured a K_i of ~10 μM for influenza viruses for HOI generated in vitro [6]. Furthermore, HOI was viricidal for SARS-CoV at unstated concentrations in another study [32]. We thus will consider 10 μM HOI as a reasonable threshold for viricidal activity of HOI for coronaviruses. Our study demonstrated that overall HOI levels in the saliva were 5.8 [3.6 – 10.5] μM (median [IQR]) after iodinated contrast exposure, compared with the baseline levels 1.9 [1.1 – 3.8] μM, indicating the possible viricidal range of HOI in the saliva after iodide load. Moreover, the higher levels of salivary HOI were sustained for at least 24 hr after iodinated contrast exposure and likely up to 48 hr, suggesting that single excess iodide load may be enough to elevate sufficient HOI levels in the saliva for several days. Although a significantly positive correlation was observed between salivary iodide and HOI levels, the slope was modest. One possible rate-limiting effect on HOI production is H_2O_2 production in the saliva. H_2O_2 is presumably provided by the salivary ductal Duox2 [10], although Duox2 expression and activity may be independent variables. In preliminary studies, we also measured H_2O_2 levels in the saliva in some samples that were detectable at low level (< 0.1 μM; data not shown), suggesting that most H_2O_2 was already consumed to produce HOI and $OSCN^-$. Therefore, the levels of Duox2 expression and activity may be the key factors determining antimicrobial oxidant production by the LPO system.

Another hypohalite, $OSCN^-$, has been extensively studied as an antimicrobial oxidant. The reported baseline salivary levels of $OSCN^-$ in 40 donors were 9.73 ± 6.62 μM (mean ± SD) measured by the most recent techniques [33]. Our study showed that overall $OSCN^-$ levels were 9.05 ± 7.34 μM (mean ± SD), consistent with the previous report. Possible viral suppressive activity of $OSCN^-$ for SARS-CoV-2 has been reported in vitro with IC_{50} ~ 11 μM [34], suggesting that salivary $OSCN^-$ levels likely attain viral suppressive levels at baseline. Higher SCN^- intake may produce higher salivary SCN^- output and $OSCN^-$ production, which may affect SARS-CoV-2 transmission, according to epidemiologic observations linking high SCN^- intake with low viral transmission [35]. Further study is necessary to compare the effects of $OSCN^-$ and HOI on SARS-CoV-2 viability.

Our data provide insight into the approximate kinetics of injected iodinated organic compounds. Generally, non-ionic iodinated contrast media contain 320-370 mg/ml of iodine as organically-bound iodine, as well as free inorganic iodide. The upper limit of free iodide in contrast media is <50 μg/ml, as stipulated by production regulations. Rendl et al reported that most formulations of modern, non-ionic contrast media contain 0.5–2.5 μg/ml free iodide [36]; thus, a single injection of 100 ml contrast medium results in 50–250 μg free iodide intravascularly. Nevertheless, a significant, rapid increase of plasma iodide levels exceeds the initial free iodide content in contrast medium, suggesting that in vivo enzymatic deiodination of organically-bound iodine in contrast medium releases free iodide into the circulation. Intravenous injection of 85 ml contrast medium (300 mg/ml iodine) with the addition of 2 or 5 μg/ml free iodide rapidly increases plasma levels of inorganic iodide to 10–15 μg/dl at ~3 min and further to ~20

μg/dl at 60 min, whereas injection of saline with 2 or 5 μg/ml free iodide alone reaches only ~2.5 μg/dl, suggesting that free iodide is rapidly released from organic molecules by deiodination [36]. Our data showed that overall salivary iodide levels after iodinated contrast exposure was 225.1 [136.6 – 495.4] μM (median [IQR]), compared with the median baseline level of 22.4 [10.7 – 36.7] μM. Based on current understanding [36], plasma levels of iodide in our study after iodinated contrast exposure are estimated to be 20 μg/dl, equivalent to 1.58 μM, thus representing a 143-fold higher iodide concentration in saliva than plasma, due to the concentrative effect of NIS localized to the basolateral membranes of the salivary intercalated and striated duct cells in humans [10,37]. Due to competition between iodine and SCN^- for transport by the NIS [12], we hypothesized that an exogenous iodine load would decrease SCN^- output and $OSCN^-$ production, a prediction not supported by the data (Fig. 1B,D). Furthermore, our data showing that the higher iodine load in the intravascularly administered contrast medium proportionally increased the salivary iodide output. These results suggest that salivary iodide levels are non-invasively obtained biomarkers for plasma iodide levels after excess iodine exposure, since the proportional relationship between plasma iodide and salivary iodide levels has been supported by us and others [38,39].

Although the population-wide use of iodine supplements is an attractive proposition, exposure to excess iodine, even as a single instance, has the potential to result in iodine-induced thyroid dysfunction that may in turn lead to end-organ damage [19]. The US Institute of Medicine advises a Tolerable Upper Limit of 1,100 μg of iodine per day [16], for which the American Thyroid Association recommends against the ingestion of any iodine supplements containing > 500 μg iodine per daily dose [40]. Our study measured the levels of chemical species in the LPO system before and after iodinated contrast exposure that even in routine medical settings has the potential of inducing thyroid dysfunction [41–44]. The effects of oral iodide supplementation with potassium iodide tablets or iodide-containing solutions, at iodine doses not exceeding the Tolerable Upper Limit [16] on the salivary LPO system should be studied. Furthermore, the direct effects of HOI or $OSCN^-$ on SARS-CoV-2 viability and transmission should be examined in future research.

Several limitations in the present study exist. First, the sample size was small. Second, the present study was strictly observational, in which the mechanism could not be investigated. Third, detailed patient-level information of the subjects was not available. Fourth, no data regarding the effect of the iodine load and salivary hypohalite concentrations on viral infection or transmission was obtained. Fifth, the colorimetric assays used to measure salivary halides and hypohalites can be subject to confounding influences, although this was addressed and controlled for in the methodology.

In conclusion, excess iodide load rapidly increases salivary output of iodide and HOI. Oral iodide supplementation in nontoxic doses may provide inexpensive and sustainable prophylaxis for airborne and aerosolized viral infections, including of SARS-CoV-2 and influenza viruses.

Abbreviations

SCN^-, thiocyanate; $OSCN^-$, hypothiocyanite; HOI, hypoiodous acid; NIS, sodium iodide symporter; LPO, lactoperoxidase; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid); β-NADPH, β-nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt hydrate; NADPI, iodinated NADPH.

Declarations

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Author contributions: Y.A., A.M.L. and J.D.K. were responsible for the study concept and design, and for drafting the manuscript. Y.A. was responsible for collection, assembly, and analysis of data. M.T.B. coordinated the study and was responsible for sample collection. R.E., J.W.C. and N.N. assisted with sample collection and manuscript editing. All authors were responsible for data interpretation.

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Data availability: All data are described in the text and depicted in the figures

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Figures

Figure 1

Salivary contents of iodide, thiocyanate (SCN^-), hypoiodous acid (HOI) and hypothiocyanite ($OSCN^-$) before and 3-6 hr after an iodinated contrast load.

Saliva was collected before (pre) and 3-6 hr after an iodinated contrast load. Salivary levels of iodide (**A**), SCN^- (**B**), HOI (**C**), and $OSCN^-$ (**D**) were measured as indicated in Methods. All data were analyzed by paired Wilcoxon matched-pairs signed rank test, two-tailed (n = 24 pairs). ****p < 0.0001.

Figure 2

Salivary contents of iodide, thiocyanate (SCN^-), hypoiodous acid (HOI) and hypothiocyanite ($OSCN^-$) before, 6 hr and 24 hr after an iodinated contrast load.

Saliva was collected before (0 hr), 6 hr and 24 hr after an iodinated contrast load. Salivary levels of iodide (**A**), SCN^- (**B**), HOI (**C**), and $OSCN^-$ (**D**) were measured as indicated in Methods. All data were analyzed by Friedman test, two-tailed, followed by Dunn's multiple comparison test (n = 16 pairs). **p < 0.01, ***p < 0.001, ****p < 0.0001 vs. 0 hr.

Figure 3

Correlations between iodine load, and salivary iodide and HOI levels

A) Paired iodine content in the injected contrast medium (iodine load) and the changes in iodide levels (Diodide) in saliva from all data were plotted (n = 39 pairs). Correlation between the iodine load and

Diodide in saliva was analyzed by the Spearman correlation test, with a simple linear regression fitted line added to the graph.

B) Paired iodide and HOI values in saliva from all data were plotted (n = 96 pairs). Correlation between iodide and HOI levels was analyzed by the Spearman correlation test, with a simple linear regression fitted line added to the graph.