Electrolytic hydrogen-generating bottle supplies drinking water with free/combined chlorine and ozone repressed within safety standard under hydrogen-rich conditions

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Abstract

Hydrogen molecules have attracted attention as a new antioxidant, but are left to be confirmedly verified whether the oral administration is highly safe or not, concurrently with retention of abundant hydrogen. When electrolysis was performed for 10 minutes using a direct-current electrolytic hydrogen-water generating bottle with tap water, "residual free chlorine" concurrently upon the production of molecular hydrogen (444 μg/L) could be appreciably decreased from 0.18 mg/L to 0.12 mg/L as quantified by a N,N-diethyl-p-phenylenediamine-dye colorimetric method. Moreover, the total chlorine concentration (residual bound chlorine plus free chlorine) was estimated to be decreased from 0.17 mg/L to 0.11 mg/L. Although a merit of electrolytic hydrogen-generating bottles exists in electrolysis for periods as short as 10 minutes, the 30-minute electrolysis brought about the more abundant hydrogen (479 μg/L) together with an oxidation-reduction potential of –245 mV; even upon this long-term electrolysis, the gross amounts of chlorine, hypochlorous acid and chloramine were shown not to be increased (0.09–0.10 mg/L from 0.11 mg/L for tap water) as detected by orthotolidine colorimetry. Above-mentioned levels of diverse-type chlorines might fulfill the World Health Organization guideline for drinking water below 5 mg/L. In addition, the dissolved ozone upon electrolytic generation of hydrogen-water was below the detection limit (< 0.05 mg/L) or undetectable, which fulfilled the official safety standards in Japan and the USA for drinking water below 0.1 mg/L, as evaluated by three methods such as an electrode-type ozone checker, indigo dye-utilizing ozone detector capillaries and potassium iodide-based colorimetry. Importantly, even when half the amount of tap water was poured into the tank of the apparatus and electrolyzed, both the residual chlorine and ozone concentrations measured were also below the safety standard. Thus, major potently harmful substances, such as residual free/bound chlorine, or hypochlorous-acid/chloramine, respectively, and dissolved ozone, as the drinking hydrogen-water was direct-current-electrolytically generated, were estimated to be repressed within safety concentration ranges with achievements of abundant hydrogen generation.

Key words: bottle; combined chlorine; electrolysis; free chlorine; hydrogen; ozone; safety standard; water

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Introduction

Gaseous bioactive molecules, such as oxygen, carbon monoxide, hydrogen sulfide and nitric oxide exert multi-functional effects on regulation of both physiological and pathophysiological aspects including cancer and cardiovascular system.

Until recently, hydrogen molecule was thought to be inactive from a viewpoint of reactivity with diverse biological macromolecules, and to have no function in mammalian cells.[1,2] However, very interestingly, it has been found that hydrogen molecules can erase the highly oxidizing substances such as hydroxyl radicals (·OH) in cells. When reactive oxygen species is produced excessively concurrently with deterioration to human intrinsic antioxidant capacity, the harmful effects of peroxidation occur, resulting in suffering from oxidative stress.

Especially, amazing and notable effects of molecular hydrogen have been found in cardiovascular emergency treatment. Attention has been focused on the effect of hydrogen molecules on the cerebral circulatory system in reducing ischemic syndrome of the brain associated with cardiac arrest. Moreover, molecular hydrogen inhalation therapy has been approved for advanced medical care by the Japanese Ministry of Health, Labor and Welfare.[3-7]

A very interesting and epoch-making advantage of using hydrogen molecule for preventive medicine is that hydrogen molecules can be an easy preparation in each household by using a water electrolysis unit. In addition, hydrogen molecules are highly safe and easy to be administered to humans, and it is thought that ingested hydrogen molecules not only enter the bloodstream to circulate throughout the body but also diffuse into each part of the body without any side effect. In general, there are three methods for introducing hydrogen molecules into the body: 1) drinking hydrogen water, 2) inhaling hydrogen gas, and 3) bathing in hydrogen water, and administration to humans is relatively easy even in ordinary households. There are still many unknowns about the mechanism of action of molecular hydrogen, and large-scale clinical-level studies are needed to verify its effects. However, ingestion of hydrogen molecule as a lifestyle is expected to be a new fundamental preventive method for many diseases caused by oxidative stress including cardiovascular disease,[3-12] cancer[13] and inflammatory disease.[14-16] Therefore,

our purpose was to elucidate the role and mechanism of action of hydrogen molecules in living organisms, and to introduce and disseminate them in lifestyle to promote health.

One concern with the spread of disease prevention using molecular hydrogen as a lifestyle is that tap water contains chlorine or hypochlorous acid to maintain sanitary safety. Further, ozone is subsidiarily generated as a harmful by-product upon electrolysis, and is also a concern because bringing out peroxidation. For the purpose of spread and recommendation of the intake of water containing hydrogen molecules for the purpose of preventing diseases, it is important to confirm the changes in the concentrations of residual chlorine and dissolved ozone in electrolyzed water accompanying electrolysis are within a safe concentration range. According to the World Health Organization guidelines for chlorine levels, a person weighing 60 kg keeps their health at 5 mg/L or less of chloride concentrations if they continue to drink 2 L of water a day daily for life.[17] The safety standard for dissolved ozone concentration in many countries, including Japan and the United States, is 0.1 mg/L.[18-19] Therefore, it is necessary to measure before and after electrolysis whether the concentration of chlorine and ozone is within the upper limit, and to carefully check from the viewpoint of hygiene and safety. In addition, the verification for the safety of household electrolyzers should be especially a measurement that is practically relevant to the lifestyle. For example, specifically, even when hydrogen molecules are generated by electrolysis using a small amount of (equivalent to a cup or a glass of) tap water for one person (corresponding to half the ruled amount of electrolyzer-equipped reservoir/tank), the safety of concentrations for residual chlorine and ozone in the electrolyzed hydrogen molecule water should also be elaborately measured and confirmed.

Thus, in the present study, the safety of drinking water generated by a domestic hydrogen molecule generator with an ultra-smooth structure in the platinum-plated surface of electrodes was examined by strictly measuring the concentrations of chlorine (free chlorine, total chlorine, and combined chlorine) and ozone concentration due to electrolysis.

Materials and Methods

Concentrations of free residual chlorine, combined residual chlorine and total residual chlorine

300 mL of tap water of Osaka City Waterworks Bureau was put into the hydrogen-dissolved water-generating electrolytic apparatus LB-002 (Lita dual hydrogen bottle, WCJ Co., Ltd., Osaka, Japan), and electrolysis was performed for 10 minutes. Other apparatuses were examined: the hydrogen water electrolyzers, Pocket (FLAX Co., Ltd., Yokohama, Kanagawa, Japan), Bliss water (World Communications Japan Co., Ltd., Osaka, Japan). After estimating the dissolved hydrogen molecule concentration using a diaphragm polarographic electrode-type hydrogen molecule concentration meter (KM2100DH, Kyoei-Electron-Lab, Tokyo, Japan), the free residual chlorine concentration and the total residual chlorine concentration were measured using a N,N-diethyl-p-phenylenediamine measurement kit (Shibata Scientific Co., Ltd., Tokyo, Japan). The combined residual chlorine concentration was calculated from the difference between the free residual chlorine concentration and the total residual chlorine concentration.[20] As another detection method, orthotolidine colorimetry was executed by utilization of the reaction of the residual chlorine which oxidized orthotolidine (Sato Shoji Co., Ltd., Kawasaki, Japan) resulting in the semi-quantitative comparison with color standard solutions as the calibration below pH 1.3 to be converted to the yellowish or yellow-brownish color.

Concentration of ozone

Dissolved ozone concentration for the hydrogen-dissolved water-generating electrolytic apparatus LB-002 was measured by using commercially available dissolved ozone detector capillary for liquid using the indigo method[21] (GASTEC No. 218). Furthermore, each concentration of these samples was strictly re-confirmed using an electrode-type dissolved ozone checker (DOC-05A, Ehara Industries Co., Ltd., Tokyo, Japan), or (DOZ-1000PE, CUSTOM Co., Ltd., Tokyo, Japan) and a colorimetric reagent by using the neutral potassium iodide method for ozone determination attached to the instrument for measuring dissolved ozone (OZ-K-1, Kasa Rika Kagaku Co., Ltd., Tokyo, Japan). In this measurement, an ozone solution generated by an electrolysis type ozone micro float for ozone water generation Ozone Associa Co., Ltd., Tokyo, Japan was quantified, diluted, and used as a standard ozone solution. All measurements were performed in duplicate, which were significantly accorded with each other.

Quantification of dissolved hydrogen concentrations and oxido-reduced potentials

The hydrogen-producing water generated by electrolysis in the hydrogen bottle was transferred to the attached beaker, stirred uniformly at a rotation speed of 990 r/min, and the dissolved hydrogen concentration in the hydrogen water measured using a diaphragm polarographic electrode-type dissolved hydrogen meter KM2100DH Kyoei Electronics Laboratory, Tokyo, Japan.

The oxidation-reduction potential was measured using an ORP meter TRUSTLEX "TL-60," TS International Co., Ltd., Hyogo, Japan.

Statistical analysis

Data were presented as the mean ± standard deviation (SD), and compared with the Student's *t*-test using Microsoft Excel 2013 for Windows (Microsoft, Seattle, WA, USA). The *P*-values below 0.01 were considered to be statistically significant. In the process of Student's *t*-test using EXCEL, it was confirmed that the histogram of the data set of this study was bell-shaped and the distribution was normal. Furthermore, a normal distribution test was performed using the reciprocal value of the standard normal cumulative distribution function of EXCEL, and it was confirmed again that the distribution was normal. All measurements were performed as $N = 4–12$ independently for 2–4 times, which significantly accorded with each other. Prior to each measurement, the measurement number was randomly assigned for each measurement, and the test measurement, control measurement, and analysis were

performed objectively by triple blinding.

Results

When electrolysis was performed using tap water for 10 minutes, no increase in the concentration of residual free chlorine associated with the production of molecular hydrogen was detected (**Table 1**). When increase in concentration for the total chlorine concentration accompanying electrolysis to generate hydrogen molecules was also measured, no increase was similarly detected. Therefore, no change was detected in the calculation result of the residual bound chlorine concentration. These results indicate that the chlorine concentration in the electrolyzed water before and after electrolysis is far below the safety standards of World Health Organization for drinking water (5 mg/L).

Furthermore, measurement and confirmation were carried out using a colorimetric reagent for measuring dissolved ozone (OZ-K-1) and an electrode-type dissolved ozone checker (DOC-05A) calibrated using the prepared standard ozone solution. As a result, before and after the electrolysis, the dissolved ozone concentration was below the detection limit (< 0.05 mg/L; **Table 1**). This indicates that the dissolved ozone concentration is lower than the safety standard concentration (< 0.1 mg/L) in many countries including Japan and the United States.

When electrolysis was performed with a Lita hydrogen bottle, the oxidation-reduction potential during electrolysis for 30 minutes was –283 mV to –220 mV, and the hydrogen molecule concentration in the apparatus was 389–579 μg/L (**Table 2**). After filling three types of hydrogen bottles of other companies with tap water and electrolyzing for 0 to 30 minutes, the concentration of free and bound residual chlorine was measured twice by the orthotolidine colorimetric method for detection of chlorine, hypochlorous acid and chloramine, and found to be time-dependently increased to 0.1, 0.2, 0.2–0.4 and 0.2–0.5 mg/L at 0, 10, 20 and 30 minutes, respectively. The reason why the measured value using the orthotolidine colorimetric method increases depending on electrolysis may be that chlorine in tap water is bonded to an organic compound more than it is evaporated as chlorine gas. On the other hand, when the Lita hydrogen bottle was filled with 330 mL of tap water and electrolyzed for 10 minutes, and the same measurement was carried out under the same conditions, no increase in the concentration of the residual chlorine in free and bound forms was found (0.1 mg/L). Furthermore, no increase in free and bound residual chlorine concentration was also observed even after generation for 20 minutes or 30 minutes (0.1 mg/L). The unchanged colorimetric values regardless lengthened electrolysis times might be attributed partly to an ultra-smooth structure in the platinum-plated surface of electrodes which causes elimination as chlorine gas into the atmosphere with no allowance for stay of chlorine- and oxygen-micro-babbles within micro-cavities of electrode surfaces.

When half the volume of water (165 mL) is electrolyzed for tap water using three types of bottles from other companies, the value was 0.2–0.35, 0.4–0.45, 0.5–1.0 mg/L in 10, 20, 30 minutes of electrolysis, respectively. On the other hand, when electrolysis of the Lita hydrogen bottle using half the volume (165 mL) of tap water was performed for 0–30 minutes, the concentrations of free residual chlorine and bound residual chlorine were almost unchanged without time-dependent increasing (0.1–0.15 mg/L). Thus, post-electrolytic amounts of the total chlorine were shown to be increased along with decreases in volumes of tap water that was poured in the bottle, but appreciably prevented for the bottle-type electrolytic hydrogen-water generator examined in the present study. Moreover, as shown in **Figure 1A** and **B**, concentrations of residual chlorine in the hydrogen molecule water generated by electrolytic apparatuses containing whole or half amount of tap water were below the safety standards (5 mg/L).

In addition, when the ozone concentration of the standard

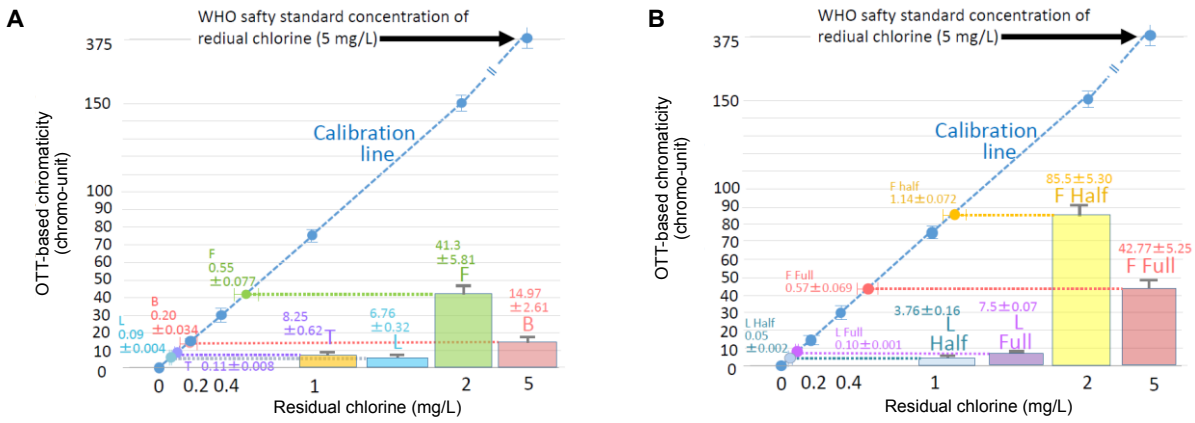

Figure 1: Concentrations of residual chlorine in the hydrogen molecule water generated by electrolytic apparatuses containing with whole or half amount of tap water.

Note: Contents of residual chlorine in the post-electrolytic hydrogen water which was prepared by diverse types of electrolysis apparatuses as evaluated by OTT-chromaticity method. (A) Osaka City-supplied Tap water was poured up to the full scale into a tank of the apparatus, and electrolyzed for 30 minutes according to the manufacturer's protocol, and then was quantified for residual chlorine by OTT method. The calibration curve was obtained using authentic chlorine, and extrapolated with OTT-to-holoquinone-conversion-based chromaticity at the absorption maximum of 435 nm for each yellowish sample. (B) Tap water was poured up to a half or full scale into the tank, and manipulated as in A. The safety standards for dissolved chlorine level (5 mg/L) according to World Health Organization guidelines are indicated by arrowhead. All concentrations of residual chlorine in the hydrogen molecule water generated by electrolytic apparatus containing with whole or half amount of tap water were below the safety standards (5 mg/L). Data are expressed as the mean ± SD, and study was executed three times. B: Bliss water; F: Flax Pocket; L: Lita dual bottle; OTT: orthotolidine; T: tap water.

Table 1: Effects of electrolysis using the hydrogen-dissolved water-generating electrolytic apparatus LB-002 (Lita hydrogen dual bottle) on concentrations of hydrogen, ozone, free residual chlorine, combined residual chlorine and total residual chlorine

Electrolysis	Hydrogen (μg/L)	Ozone (mg/L)	Free residual chlorine (mg/L)	Combined residual chlorine (mg/L)	Total residual chlorine (mg/L)
Before electrolysis	< 0.005	N.D. (< 0.05)	0.18±0.03	0.17±0.03	N.D.
10 min	444±7.2	N.D. (< 0.05)	0.12±0.02*	0.11±0.02**	N.D.

Note: Date are expressed as the mean ± SD (n = 12). The examinations were four times executed, which substantially accorded with each other. *P = 0.0006, **P = 0.0005 (Student's t-test). N.D.: Not detected.

Table 2: The oxidation-reduction potential and the hydrogen molecule concentration in electrolyzed water generated by use of electrolytic apparatus LB-002 (Lita hydrogen dual bottle) with electrolysis of tap water for 30 minutes

	Oxidation-reduction potential (mV)	Hydrogen molecule concentration (μg/L)
Before electrolysis (tap water)	+267.0±3.3	0.3±0.4
After electrolysis		
1	–283.8±20.9	579.0±30.6
2	–241.5±59.3	473.8±22.1
3	–219.5±38.3	389.0±11.0
4	–236.8±7.9	475.3±72.9
Mean±SD (all 16 samples of 1–4)	–245.4±43.9	479.3±79.1

Note: Data are expressed as the mean ± SD (n = 4). Four independent measurements were executed for each lot apparatus differently manufactured.

ozone solution generated as the positive control was measured, the measured values of the indigo dye-utilizing ozone detector capillaries, the potassium iodide-based colorimetry and the measured values using the electrode-type ozone checker were all close to one another. Importantly, the concentrations of ozone in the hydrogen molecule water generated by the electrolytic apparatus containing with whole or half amount of tap water were below the safety standards for dissolved ozone concentration (0.1 mg/L) in many countries including Japan and the United States (**Figure 2**). Overall, this research confirmed the safety for both of residual chlorine and ozone in electrolyzed water even when hydrogen molecules are generated by electrolysis using a small amount (165 mL) of (equivalent to a cup or a glass of) tap water for one person (corresponding to half the amount of electrolysis equipment).

Discussion

Regarding the effects of molecular hydrogen, in addition to the accumulation of evidence obtained from experimental animal models, clinical trials have been conducted and are still ongoing.[1,2,8-12] Hydrogen molecules have been thought to behave as the inert gas in mammalian cells, and it has traditionally been thought that hydrogen molecules do not react with oxygen-containing biological compounds at room temperature without a catalyst.[1,2] In 2007, it was shown that hydrogen molecule eliminates reactive oxygen/nitrogen species such as

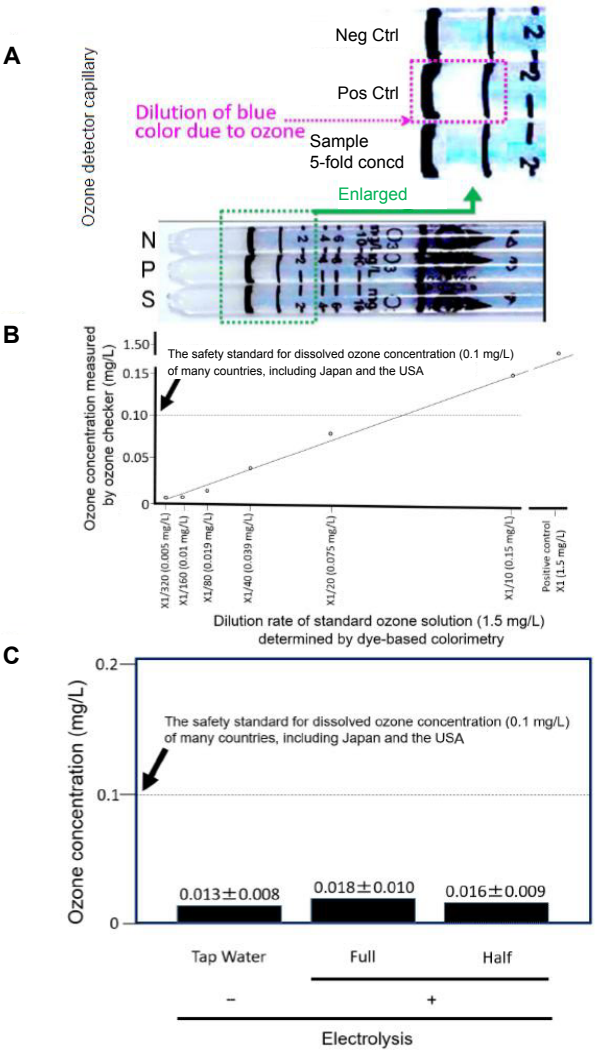

Figure 2: Ozone concentrations in the hydrogen molecule water generated by electrolytic apparatus LB-002 (Lita hydrogen dual bottle) with whole or half amount of tap water.

Note: (A, B) Data were calibrated and confirmed in the same way as Table 1 using a combination of the dissolved ozone detector capillaries for liquids using the indigo method and the potassium iodide-based colorimetric method. When it was measured using ozone detector capillary that produces isatin being almost colorless by reaction of ozone (with the detection agent indigo blue dye and changes whitish), the position of the changed colorless compound was shown by a magenta colored dashed square since the difference between the original color (light blue) and the changed color (slightly whitish) was feasible to distinguish on the photograph. (C) Whole amount (330 mL) (full) or half amount (165 mL) of tap water was electrolyzed for 10 minutes, and concentrations of ozone were measured by an electrode-type dissolved ozone checker DOZ-1000PE. The safety standards for dissolved ozone concentration (0.1 mg/L) in many countries including Japan and the United States, are indicated by dashed lines (B and C). Data are expressed in the bar chart as the mean ± SD (measured 3 times at n = 4).

hydroxyl radicals (·OH) and ($ONOO^-$) in cells.[1] In the human body, reactive oxygen species are routinely produced as a by-product of energy metabolism by oxidative phosphorylation. In addition, excessive reactive oxygen species is additionally produced by smoking, air pollution, exposure to ultraviolet and radiation, intense exercise, physical and psychological stress, and so on. In addition, it should be noted that some chlorine compounds and ozone are also strong oxidants that are harmful to human health. Therefore, when drinking the tap water by electrolysis, it is extremely important and indispensable to confirm the safety of electrolyzed water containing generated hydrogen molecules.

Chlorine has an atomic number of 17 and has 7 electrons in the outermost-shell electron orbit. Therefore, chlorine is unstable and highly reactive, and readily forms another compound by reaction with other elements. In addition, when electrons are received from the cathode during electrolysis and highly reactive chlorine molecules and chlorine compounds are newly generated in the electrolyzer, there is a concern that they may be highly toxic to living organisms. Therefore, it is necessary to check the residual concentrations of chlorine, total chlorine and combined chlorine. Tap water is required to retain free chlorine or bound chlorine for sanitary management, and chlorine was actually detected before electrolysis. However, no increase was seen after electrolysis.

In single-tank electrolyzers such as examined in the present study, when chloride compounds such as sodium chloride, potassium chloride, and hydrochloric acid are present, chlorine is newly generated by electrolysis. However, no increase in chlorine was observed in the present study. Therefore, it was considered that the tap water used in the present study did not contain any chlorine compounds that would generate new chlorine by electrolysis, and almost no chlorine compounds were eluted into the electrolytic water from the devices that was energized during the electrolysis.

When electrolysis is carried out on both sides of an electrolyte membrane (a membrane having physical properties of passing only ions without passing electrons) between the anode and the cathode, generation of ozone from the anode is concerned. However, no ozone was detected in the present study. Since the present apparatus is a single-tank type in which the anode and the cathode are not separated by an electrolyte membrane named “a diaphragm,” it was considered that the generation of ozone disappeared or was immediately decomposed to be below the detection limit.

In conclusion, the present study shows the safety of dissolved chlorine and ozone concentrations in electrolytic hydrogen-water generators containing molecular hydrogen from domestic electrolysis, which is considered to be effective in promoting healthy lifestyles and health promotion. Moreover, this innovative research indicates that it is possible to supply hydrogen molecules within the range of safety standards for chlorine and ozone daily by using a household electrolyzer with an ultra-smooth structure in the platinum-plated surface of electrodes, which causes elimination as chlorine gas into the atmosphere, without allowance for stay of chlorine- and oxygen-microbubbles within micro-cavities of electrode surfaces.

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Author contributions
TH wrote the initial draft, and TH and NM completed the final draft. Both authors read and approved the final manuscript for publication.
Conflicts of interest
There is no conflict of interest.
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