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In Vivo Nanoelectrospray for the Localization of Bioactive Molecules in Plants by Mass Spectrometry

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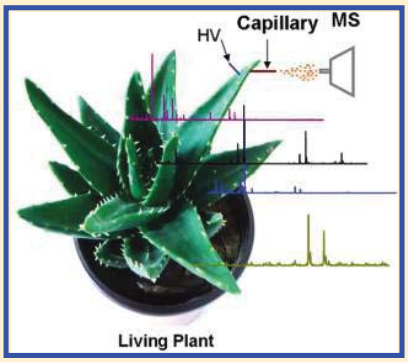
Supporting Information

ABSTRACT: The method for the localization of bioactive molecules in plants is highly needed since it provides a fundamental prerequisite for understanding their physiological and ecological functions. Here, we propose a simple method termed in vivo nanoelectrospray for the localization of bioactive molecules in plants without sample preparation. A capillary is partly inserted into the plant to sample liquid from a highly located region, and then, a high voltage is applied to the plant to generate an electrospray from the capillary tip for mass spectrometry analysis. Using this method, bioactive molecules such as saccharides, glycoalkaloids, flavonoids, organic acids, and glucosinolates (GLs) are detected in the target regions of living plants or fresh fruits. Original information for endogenous chemicals including liable molecules in plant can be obtained. A sketchy three-dimensional distribution of glycoalkaloids in a cherry tomato has been obtained. The present work provides a powerful tool for the study of bioactive molecules in a living plant by mass spectrometry.



Some of the plant-derived bioactive molecules are signaling molecules for the regulation of the growth, development, and reproduction of plants,[1,2] as well as the plant interactions with outside environments.[3−5] Studies on the localization of these bioactive molecules in plant cell or tissues provide fundamental information for understanding their physiological and ecological function.[6−8] Mass spectrometry (MS) is an attractive tool for bioactive molecule analysis because of its high selectivity and sensitivity.[9] To obtain the information of endogenous compounds in different parts, plant samples have to be cut into small pieces for solvent extraction separately and then analyzed by conventional electrospray ionization (ESI)-MS.[10] However, most location information would be lost during the sample preparation process. Ambient ionization methods[11−13] including desorption electrospray ionization (DESI)[14] and nanospray desorption electrospray ionization (nano-DESI)[15] are available for directly obtaining molecular localization information in biological tissue with little sample preparation. As these methods are a surface analysis technique, a serial section has been applied to obtain the subsurface information of specific molecules.[16,17] However, in the serial sectioning approach, serial sections of the sample are obtained through mechanical sectioning and are individually analyzed. The intact tissue structure may be damaged and exposed to the atmosphere, which may result in the degradation of molecules.[18] Leaf spray mass spectrometry,[19] a variant of paper spray,[20,21] is a powerful method which is capable of providing real-time molecular information of an intact plant. However, a small nick has to be cut in the plant to form a sharp tip before electrospray, which makes it difficult to obtain the original spatial distribution information of bioactive molecules in the plant.

Herein, we propose a simple ESI-based method termed in vivo nanoelectrospray, which is capable for obtaining the original localization information of bioactive molecules in plants without sample preparation. The in vivo nanoelectrospray can be regarded as the combination of capillary effect[22−24] and nanoelectrospray ionization,[25,26] which is similar to the scanning mass spectrometry (SMS) probe.[27] The method has two major features in that: (1) it can have in situ analysis in the inner part of the plant including a living plant without sample preparation, so as to obtain the original information of bioactive molecules, and (2) it has a satisfactory resolution due to the low sample consumption (nanoliters volume), which enables one to acquire the spatial distribution information of specific molecules in a plant. We demonstrate the method for the localization of bioactive molecules in different plant samples. Bioactive molecules including saccharides, glycoalkaloids, flavonoids, organic acid, and intact glucosinolates (GLs) have been detected in different localizations of plants, and a sketchy three-dimensional (3-D) distribution of glycoalkaloids in a tomato has also been conveniently obtained.

EXPERIMENTAL SECTION

The in vivo nanoelectrospray experiment is illustrated in Figure 1a. Plant samples (cherry tomato, grape, kumquat, radish, and potted aloe) are purchased from the local market without any special treatment. The sample is placed on an electrically

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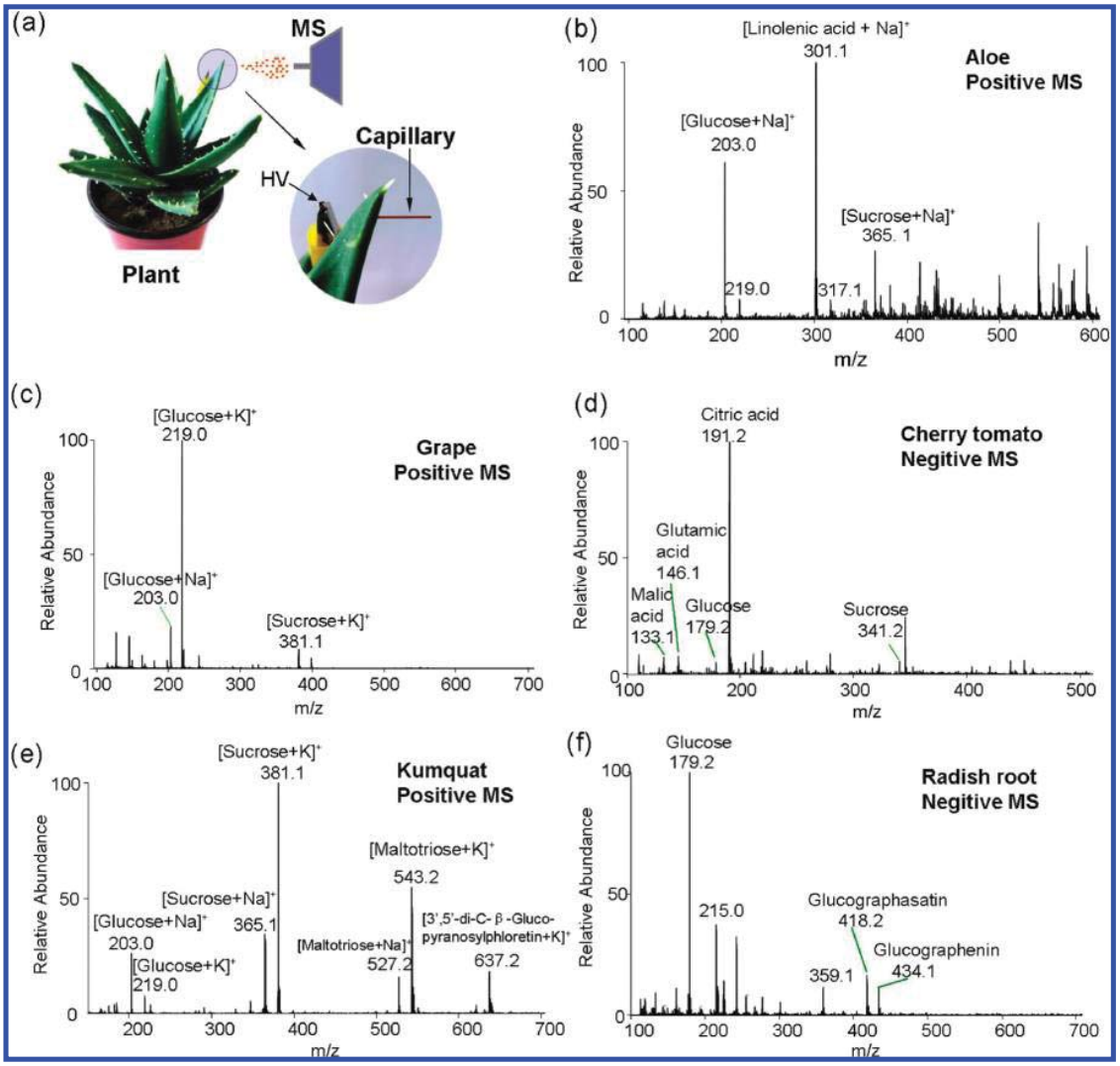

Figure 1. (a) Schematic diagram of in vivo nanoelectrospray. Mass spectra acquired from (b) living aloe in positive ion mode, (c) grape fruit in positive ion mode, (d) cherry tomato fruit in negative ion mode, (e) kumquat fruit in positive ion mode, and (f) white radish root.

insulative platform. A quartz capillary (50 μm i.d., 148 μm o.d.) located in front of the inlet of the mass spectrometer is inserted into the plant, and due to the capillary effect, the liquid flows from the capillary end inside the plant to the end outside. A stainless needle is inserted into the plant. A high DC voltage (3.5 kV, positive or negative) is applied to the needle to generate a spray of charged droplets carrying bioactive molecules toward the inlet of mass spectrometer. All MS and MS/MS measurements are carried out using a Thermo LTQ mass spectrometer (Thermo Scientific, San Jose CA). The capillary temperature is set to 275 ° C, capillary voltage is set to 9 V, and the tube lens voltage is set to 100 V. Unless otherwise specified, all mass spectra are obtained by averaging the data acquired in a time window of 2.4 s (24 scans). The distance between the capillary end outside and the MS inlet is 30 mm, and the length of capillary outside the plant is 10 mm.

During the conventional ESI-MS experiment, a piece of plant sample is loaded in a 2 mL vial. After crushing and centrifugation, the supernatant liquid is collected and analyzed by ESI-MS. Liquid sample is directly injected into the loop (20 μL) for electrospray ionization. Electrospray solvent is methanol/water (1:1) with a flow rate 200 μL/min.

RESULTS AND DISCUSSION

The in vivo nanoelectrospray involve the process of directly drawing the liquid from the plants by a capillary and ionizing the samples with electrospray. Living and fresh plant samples including potted aloe, intact fruits, and radish roots are used to verify the feasibility of this method. A quartz capillary is inserted into the aloe leaf to draw out the liquid from target regions, and subsequently, an electrical potential is applied to the plant to perform electrospray and MS analysis (Figure 1a). In the mass spectrum of aloe leaf, sodium and potassium adducts of linolenic acid (m/z 301.1 [linolenic acid + Na]$^+$ and m/z 317.1 [linolenic acid + K]$^+$) are the most abundant ions observed (Figure 1b). With similar method, saccharides, organic acids, and flavonoids are detected from fruit samples. In the positive ion mode, the mass spectrum of grape (Figure 1c) is dominated by cationized sugars (m/z 203.0 [glucose + Na]$^+$, m/z 219.0 [glucose + K]$^+$, m/z 365.1 [sucrose + Na]$^+$, and m/z 381.1 [sucrose + K]$^+$). In the negative mode, the mass

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spectrum of cherry tomato contains amino acids (m/z 146.1 [glutamic acid − H]$^-$), organic acids (m/z 133.0 [malic acid − H]$^-$ and m/z 191.0 [citric acid − H]$^-$), and saccharides (m/z 179.2 [glucose − H]$^-$ and m/z 341.2 [sucrose − H]$^-$) are detected as deprotonated molecules (Figure 1d). In addition to monosaccharide and disaccharide, trisaccharides (m/z 527.2 [maltotriose + Na]$^+$ and m/z 543.2 [maltotriose + K]$^+$) and flavonoid (m/z 637 [3′,5′-di-*C*-β-glucopyranosylphloretin + K]$^+$)[28] are observed in the mass spectrum of kumquat in positive ion mode (Figure 1e). The potassium adducts (m/z 637.2) are identified by MS/MS measurement[29] (Figure S1, Supporting Information). Extracts of these fruits are analyzed by conventional ESI-MS for comparison purposes. Mass spectra of fruit samples produced by conventional ESI-MS (Figure S2, Supporting Information) are dominated by the same cationized saccharides or deprotonated acids as shown in Figure 1. Besides living aloe and fresh fruits, bioactive molecules are also detected in radish root. Two intact glucosinolates (GLs) are observed as deprotonated molecules ions in the mass spectrum of fresh radish, which are [glucoraphasatin − H]$^-$ (m/z 418.2) and [glucoraphenin − H]$^-$ (m/z 434.1) (Figure 1f). It should be pointed out that the chemical profiles are biased toward more polar components since the present methods are ESI-based techniques.

The in vivo nanoelectrospray method can obtain the original molecular information from an intact plant, because the sampling and ionization are nearly in the same time. Some bioactive molecules in plant are subject to change during the sample preparation. For example, GLs in fresh radish are sulfur-rich bioactive molecules commonly existing in many agriculturally important crops (cabbage, cauliflower, horseradish, turnip, mustard and rapeseed, etc.). Disruption of cellular structures of plants by cutting, chewing, cooking, and freezing may result in hydrolysis of GLs.[18] We test different points in a small part of fresh radish root randomly by in vivo nanoelectrometry mass spectrometry, and GLs including glucoraphenin (m/z 418.2) and glucoraphasatin (m/z 434.1) are observed in all the mass spectra (Figure 2a). However, the peaks of glucoraphenin and glucoraphasatin are absent in the mass spectrum of conventional ESI-MS (Figure 2b), during which the same part of radish is pretreated with crashing and centrifugation.

The in vivo nanoelectrospray can also perform microregions analysis since the inner diameter of capillary is small. The liquid volume needed for transport and spray is at a nanoliter scale. We select kumquat as the sample to perform the microregions analysis since kumquat has been commonly employed in folk medicine, and some bioactive compounds in kumquat rind have been studied for their pharmacological activity.[30] Kumquat rind is smooth with a number of little oil glands (0.3−1.0 mm diameter) on it (Figure 3A). Saccharides such as glucose (m/z 219.0), sucrose (m/z 365.1 and 381.1), and maltotriose (m/z 543.2) are detected as the major peaks in the mass spectrum of the oil gland (Figure 3B). However, mass spectra obtained of the shiny skin outside the oil glands are totally different, which are dominated by protonated catechin (m/z 291.2 [catechin + H]$^+$) and potassium adducts of catechin (m/z 329.2 [catechin + K]$^+$) (Figure 3C). Catechin is a flavonoid that is found to have antioxidant, anticancer, and anti-inflammatory effects.[31,32] The experimental data reveals that chemical compositions varied significantly in different regions of the kumquat rind, which can be identified by in vivo nanoelectospray mass spectrometry.

Besides obtaining the information of bioactive molecules in the highly located microregion of plant, the in vivo nanoelectrospray can also perform 3-D spatial distribution analysis of specific molecules inside the plant. Glycoalkaloids in tomato are reported to be involved in host−plant resistance and to have a variety of pharmacological and nutritional properties in animals and humans.[33] Lycoperoside F is a kind of glycoalkaloids present in different tissues of tomato with different concentrations.[34,35] Herein, we obtained the spatial distribution of lycoperoside F in cherry tomato by in vivo nanoelectrospray mass spectrometry.

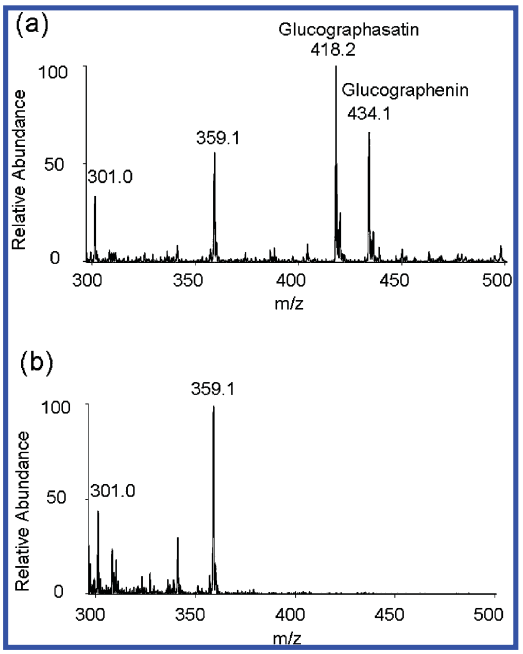

Figure 2. Mass spectra of radish obtained from (a) in vivo nanoelectropray mass spectrometry and (b) conventional ESI-MS in negative ion mode.

During the experiment, the capillary is inserted into the cherry tomato vertically with inner depth of 2 mm as a start point. The inserted depth of the capillary is increased gradually by an increment of 2 mm step-by-step until the capillary reached the core of cherry tomato. Nine test points (a∼i) are distributed on the surface of cherry tomato, from which the capillary is inserted into the tomato. At last, mass spectra data obtained from different points are collected. Figure 4A show the positive ion mass spectrum of the point d with depth of 6 mm. The mass spectrum is dominated by protonated lycoperoside F molecule (m/z 1270.5 [M + H]$^+$), which is identified by MS/MS measurement (Figure 4A, inset). The MS/MS spectrum shows the fragment ion peaks [M + H − H_2O]$^+$ at m/z 1252.3, [M + H − AcOH]$^+$ at m/z 1210.5, [M + H − H_2O − Glc]$^+$ at m/z 1090.6, [M + H − AcOH − Glc]$^+$ at m/z 1048.5, [M + H − H_2O − AcOH − Glc]$^+$ at m/z 1030.4, [M + H − AcOH − Xyl − 2Glc]$^+$ at m/z 754.4, and [M + H − AcOH − Xyl − 2Glc − Gal]$^+$ at m/z 592.3. Figure 4B gives the 3-D spatial distribution of lycoperoside F in cherry tomato. The height of the cone represents the signal-noise ratio (S/N) value of the peak at m/z 1270.5 in the mass spectrum of the test point. The x axis represents the depth that the capillary inserts into the cherry tomato. According to the difference of S/N value, tomato can be divided into three layers, the yellow area (0−4 mm), the blue layer (4−14 mm), and the white area (14−16 mm). Lycoperoside F is not detected in the inner core of the tomato. However, the content of lycoperoside F is

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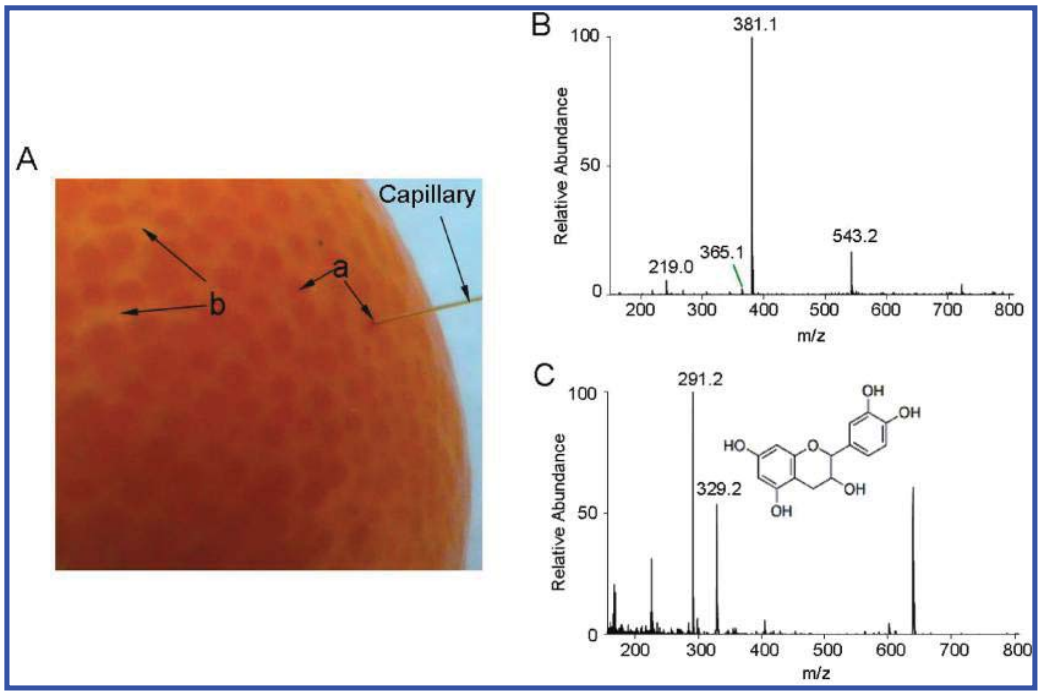

Figure 3. Localization of bioactive molecules in kumquat rind performed by in vivo nanoelectrospray mass spectrometry. (A) Photograph of kumquat, in which region a contains the oil gland and region b is the skin outside the oil gland. (B) Mass spectrum obtained from the region a in kumquat rind with 1 mm depth. (C) Mass spectrum acquired from the region b in kumquat rind with 1 mm depth.

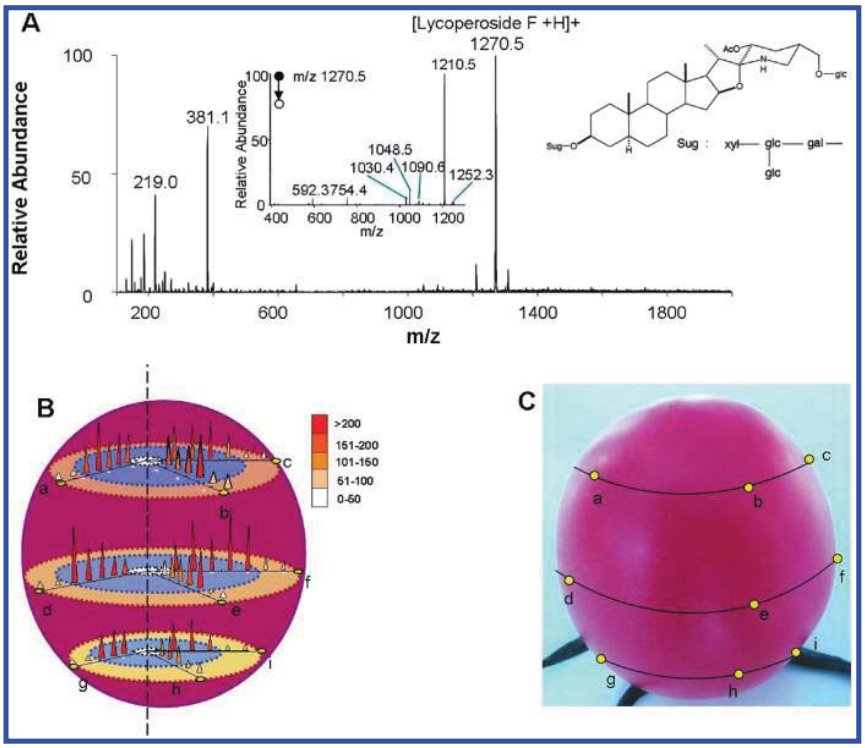

Figure 4. Localization lycoperoside F in cherry tomato performed by in vivo nanoelectrospray mass spectrometry. (A) The positive ion mode mass spectrum of point d with the depth of 6 mm. (B) The spatial distribution of lycoperoside F in cherry tomato; height of the cone represents the S/N value of the peak at m/z 1270.5. Points (a∼i) are represent the points on the surface of tomato from where capillary is inserted into the cherry tomato; the distance between adjacent two points in the same line is 2 mm. (C) Photograph of cherry tomato, and the cherry tomato diameters of layer of a−b−c, d−e−f, and g−h−i are 28, 32, and 24 mm, respectively.

abundant in the middle layer and significantly decreases in the outer layer. Their relative mass spectra are showed in Figure S3, Supporting Information. Actually, the three parts are correlated with the different tissues within the tomato, which are pericarp

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(outer layer), jelly parenchyma (middle layer), and columella (inner core). Moco et al. have also analyzed the different areas of tomato using a conventional method of "section-extraction-MS analysis" and revealed that lycoperoside F is present particularly in jelly parenchyma.[34]

CONCLUSIONS

The in vivo nanoelectrospray mass spectrometry for the localization of bioactive molecules has been performed with various plant samples. This method can obtain the original information of the labile ingredient in the plant. With a small liquid volume consuming, it is able to analyze microregions inside the plant, as well as obtain the 3-D spatial distribution of specific molecules. The method is applicable for the analysis of various samples including living plants and other organisms containing enough liquid. Since many of the bioactive molecules in plant cell are of great interest in plant physiology research,[36−39] the application of the present method to a single plant cell analysis will be the next goal. However, it will be very challenging since it requires strategies for improving the resolution and implementation of the system to accurately control capillary-tip/sample relative position.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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