QJM: An International Journal of Medicine, 2020, 870–875

doi: 10.1093/qjmed/hcaa164
Advance Access Publication Date: 14 May 2020
Original paper

ORIGINAL PAPER

Hydrogen gas (XEN) inhalation ameliorates airway inflammation in asthma and COPD patients

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Summary

Background: Hydrogen was proven to have anti-oxidative and anti-inflammation effects to various diseases.
Aim: We wish to investigate the acute effects of inhaled hydrogen on airway inflammation in patients with asthma and chronic obstructive pulmonary disease (COPD).
Design: Prospective study.
Methods: In total, 2.4% hydrogen containing steam mixed gas (XEN) was inhaled once for 45 min in 10 patients with asthma and 10 patients with COPD. The levels of granulocyte-macrophage colony stimulating factor, interferon-γ, interleukin-1β (IL-1β), IL-2, IL-4, IL-6 and so on in peripheral blood and exhaled breath condensate (EBC) before and after 'XEN' inhalation were measured.
Results: 45 minutes 'XEN' inhalation once decreased monocyte chemotactic protein 1 level in both COPD (564.70–451.51 pg/mL, $P = 0.019$) and asthma (386.39–332.76 pg/mL, $P = 0.033$) group, while decreased IL-8 level only in asthma group (5.25–4.49 pg/mL, $P = 0.023$). The level of EBC soluble cluster of differentiation-40 ligand in COPD group increased after inhalation (1.07–1.16 pg/mL, $P = 0.031$), while IL-4 and IL-6 levels in EBC were significantly lower after inhalation in the COPD (0.80–0.64 pg/mL, $P = 0.025$) and asthma (0.06–0.05 pg/mL, $P = 0.007$) group, respectively.
Conclusions: A single inhalation of hydrogen for 45 min attenuated inflammatory status in airways in patients with asthma and COPD.

Introduction

Hydrogen, identified as antioxidants, in particular have been shown to have distinct characteristics, including its effect on specific reactive oxygen species (ROS) and excellent diffusion capacity.[1] It has been demonstrated that hydrogen could provide protection against various diseases, including sepsis, stroke and ischemia-reperfusion injury.[2,3] The major feature of chronic obstructive pulmonary disease (COPD) and other airway

Received: 18 March 2020; **Revised (in revised form):** 1 May 2020

diseases is generally regarded as abnormal response to injury, chronic inflammation, excessive activation of macrophages, neutrophils, T lymphocytes and fibroblasts in the lung and oxidative stress is widely proposed as a pathogenic mechanism while ROS plays a pivotal role in the incidence and exacerbation of diseases.[4] It is still unknown, however, whether hydrogen with low concentration inhalation has a therapeutic role on human diseases with airflow limitation. The purpose of this study was to investigate the effect of inhaled hydrogen gas on airway inflammation in patients with COPD and asthma.

Methods

Subjects

From March 2019 to June 2019, 10 COPD and 10 asthma patients (aged 20–65 years old) were recruited to participate in this study in Peking Union Medical College Hospital. COPD patients were restricted to those with spirometrically confirmed airflow obstruction (postbronchodilator forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) < 0.7).[5] Asthma patients met the diagnostic criteria of asthma.[6] Oxygen saturation on resting-state using pulse oximetry and Medical Research Council (MRC) scores for dyspnea were recorded.[7] Exclusion criteria included pregnancy, breast feeding, symptoms of acute airway infections or exacerbation 4 weeks prior to the test, and past history of malignancy, myocardial infarction, liver cirrhosis, renal failure and mental or intellectual disorders who were judged to not be able to provide informed consent.

The study protocol was reviewed and approved by the ethical committee of the Peking Union Medical College Hospital (HS-1948), and the protocol was carried out in accordance with relevant ethical guidelines and regulations. The written consents were obtained from all subjects.

Pulmonary function

Pulmonary function was measured by FEV1, FVC, FEV1/FVC and bronchial provocation test used a MasterScreen spirometer (CareFusion, Hoechberg, Germany). All measurements were performed according to the standards established by the American Thoracic Society.[8]

Hydrogen gas administration

A machine developed by Earth Engineering Co. (Suisonia, FRJ-003, Kitakyushu, Japan) was used to decompose superheated steam to produce a mixed gas containing hydrogen (H_2) gas. The stream produced by heating sterile water for inhalation, and it decomposes into H_2 and oxygen (O_2) at a decomposition ratio of 67% vs. 33%. As air is present inside the machine, the concentration of H_2 gas is ~2.4%, according to the Manufacture's instruction confirmed by a portable type hydrogen detector. After transfer through nasal cannula the H_2 concentration is ~0.1–0.3% when inhaled, while O_2 is adsorbed with a cartridge. This steam mixed gas is designated as 'XEN' in preliminary study. All subjects performed the hydrogen gas inhalation for 45 min under close observation of researchers to ensure the compliance and to find any adverse reactions.

Exhaled breath condensate (EBC) from all participants was obtained according to the American Thoracic Society/European Respiratory Society guidelines using RTube™ (Respiratory Research, Inc, Austin, TX, USA) by breathing tidally into the device precooled to −20°C. Before and after 45 min of 'XEN' inhalation, peripheral blood and EBC were collected and storage in −80°C.

Reagents and measurement

MILLIPLEX MAP assay beads are comprised of polystyrene microspheres that have been impregnated with ferrite particles as well as a mixture of two colored dyes. The level of granulocyte-macrophage colony stimulating factor, interferon-γ, interleukin-1β (IL-1β), IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-17A, macrophage inflammatory protein-1α (MIP-1α), MIP-1β, tumor necrosis factor alpha (TNF-α) were detected by multiplex analysis using T Cell Magnetic Bead Panel (HSTCMAG-28SK-14) and macrophage-derived chemokine, soluble cluster of differentiation-40 ligand, monocyte chemotactic protein 1 (MCP-1), vascular endothelial growth factor A using Human Cytokine/Chemokine Magnetic Bead Panel (HCYTOMAG-60K-04) (EMD Millipore Corp, Billerica, MA, USA). Luminex® xMAP® technology was used for detection and analysis concentrations of multiple target cytokine in a single sample, as recommended by the manufacturer. Sandwich enzyme-linked immunosorbent assay was used to measure the level of human superoxide dismutase 3 (SOD3) using LF-EK0107 kit (AbFrontier, Seoul, South Korea).

Statistical analysis

Statistical analysis was performed using SPSS 19.0 software (SPSS Inc, Chicago, IL, USA). Values were presented as the mean ± the standard deviation and the Shapiro–Wilk Test was used to assess the normal distribution of data. To compare the mean of a single group before and after 'XEN' inhalation, paired samples t test after taking natural logarithms were performed and statistical significance was established at $P < 0.05$.

Results

The characteristics of the subjects

The characteristics of the 20 subjects are listed in Table 1. Patients with COPD were older, with more male participates than asthma group. Pulmonary function showed COPD patients had lower levels in FEV1, FVC and FEV1/FVC compared with asthma patients ($P < 0.05$).

Measurement of inflammatory factors

Regarding the proinflammatory mediators in peripheral blood, MCP-1 level in both COPD (564.70–451.51 pg/mL, $P = 0.019$) and asthma (386.39–332.76 pg/mL, $P = 0.033$) group decreased significantly after inhalation (Figure 1a), while IL-8 level decreased only in asthma group (5.25–4.49 pg/mL, $P = 0.023$, Figure 1b). In the case of CD40L in COPD group, the level in EBC increased after inhalation (1.07–1.16 pg/mL, $P = 0.031$). However, IL-4 and IL-6 level in EBC was significantly lower after inhalation in the COPD (0.80–0.64 pg/mL, $P = 0.025$) and asthma (0.06–0.05 pg/mL, $P = 0.007$) group, respectively, compared with the data before inhalation (Figure 2). There was no significant difference in SOD3 level before and after inhalation (Table 2).

Discussion

Hydrogen, which exhibits anti-oxidative and anti-inflammation effects, was proved to be relatively safe for inhalation in diving.[9,10] The concentration of hydrogen used in our study is quite

Table 1. Characteristics of the study population

	COPD (N = 10)	Asthma (N = 10)	P value
Age, years (range)	61.9±5.9 (52–70)	46.8±13.3 (21–64)	0.006*
Sex (M/F)	10/0	3/7	0.001*
BMI (kg/m^2)	25.30±3.02	24.32±3.01	0.499
Pack-years	36.9±12.9	8.2±7.2	0.001*
Oxygen saturation (%)	95.0±1.9	96.3 ±1.1	0.098
mMRC of dyspnea	3.3±1.0	1.7±0.8	0.001*
FEV_1 (%pred)	46.08±16.21	74.32±27.34	0.016*
FVC (%pred)	79.50±13.45	96.36±12.87	0.014*
FEV_1/FVC, %	45.55±13.58	74.06±15.10	0.001*

Sex ratio were compared using Pearson Chi-square test. Quantitative data are expressed as mean ± SD, and *P* values were obtained by the independent sample *t* test.
BMI: body mass index; COPD: chronic obstructive pulmonary disease; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; mMRC: modified Medical Research Council.
*Values indicate significant differences ($P < 0.05$).

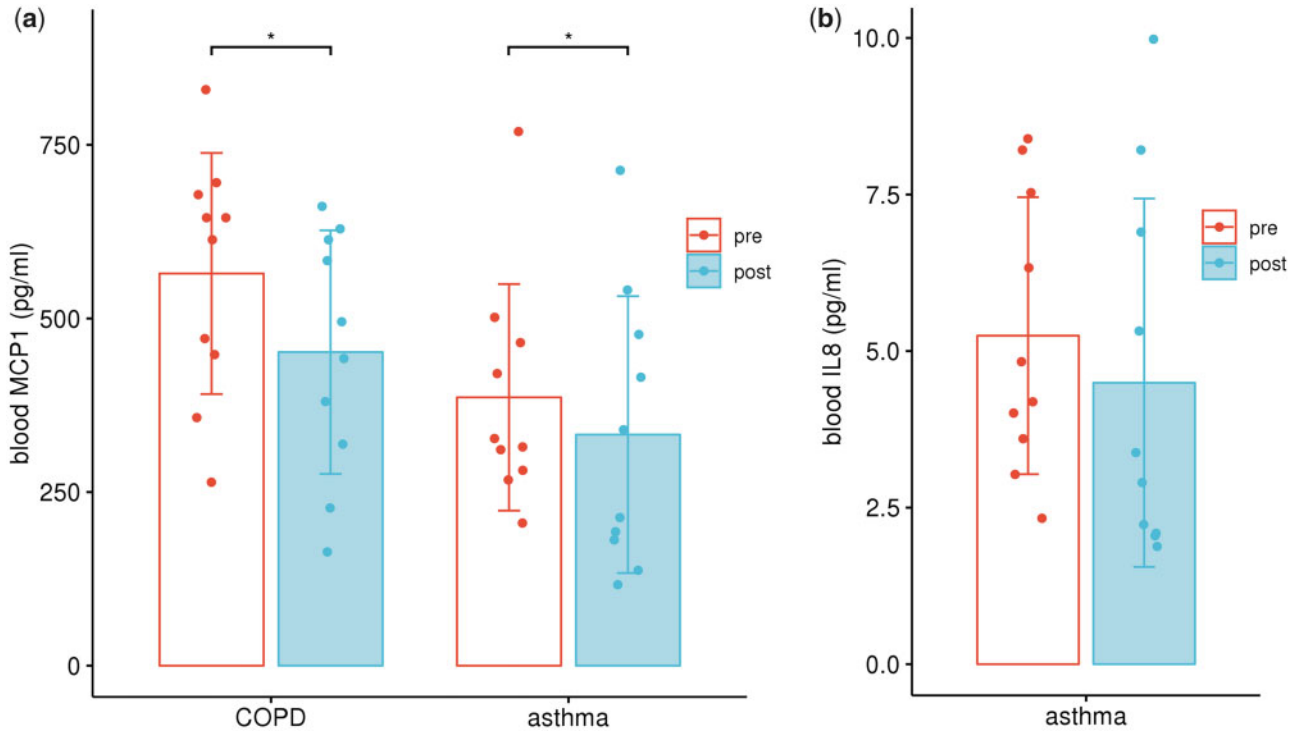

Figure 1. Blood cytokine levels before and after hydrogen inhalation. (a) MCP-1 level decreased after hydrogen inhalation in both COPD and asthma group; (b) IL-8 level decreased significantly after hydrogen inhalation in asthma group. *P < 0.05, paired t test.

lower (~2.4%). Due to its small molecular weight, hydrogen can easily penetrate bio-membranes and diffuse into cytosol and organelles, and the tissue compatibility of hydrogen is stronger than many other oxidant scavengers. In this study, we demonstrated that a single inhalation of 45 min hydrogen gas could reduce airway inflammatory mediators in patients with asthma or COPD.

Antioxidants may be effective in the protection against the damage of oxidative stress from trauma or infection. Molecular hydrogen specifically quenches detrimental ROS (·OH), while maintaining the metabolic oxidation–reduction reaction and other less potent ROS, such as hydrogen peroxide (H_2O_2), $O_2^{\cdot-}$ and nitric oxide(NO·). Ohsawa *et al.*[1] demonstrated that 2% hydrogen inhalation can alleviate oxidative stress by selectively neutralizing hydroxyl radicals (·OH) and antagonizing peroxynitrite (ONOO−), and proved to be effective for other cytotoxic ROS-related diseases.[2,11] SOD is an important antioxidant enzyme *in vivo*, which can scavenge superoxide radical and decompose them into low-activity H_2O_2. Hydrogen was proved to increase the expression of antioxidative enzymes such as NF-E2-related factor 2 (Nrf2) and SOD, subsequently ameliorating oxidative stress and inflammatory responses.[12,13] Huang *et al.*[14] found that 42% hydrogen inhalation enhanced alveolar macrophage phagocytosis in ovalbumin-induced asthmatic mice, which may be associated with the antioxidant effects of hydrogen and the activation of the Nrf2 pathway, that significantly alleviated airway hyperresponsiveness, inflammation and goblet cell hyperplasia, diminished type-2 helper T-cell (TH_2) response, malondialdehyde (MDA) production, decreased IL-4 and immunoglobulin E levels and increased SOD activity.

In our study, we used SOD3 level to evaluate the antioxidative effect and found the level did not change significantly after

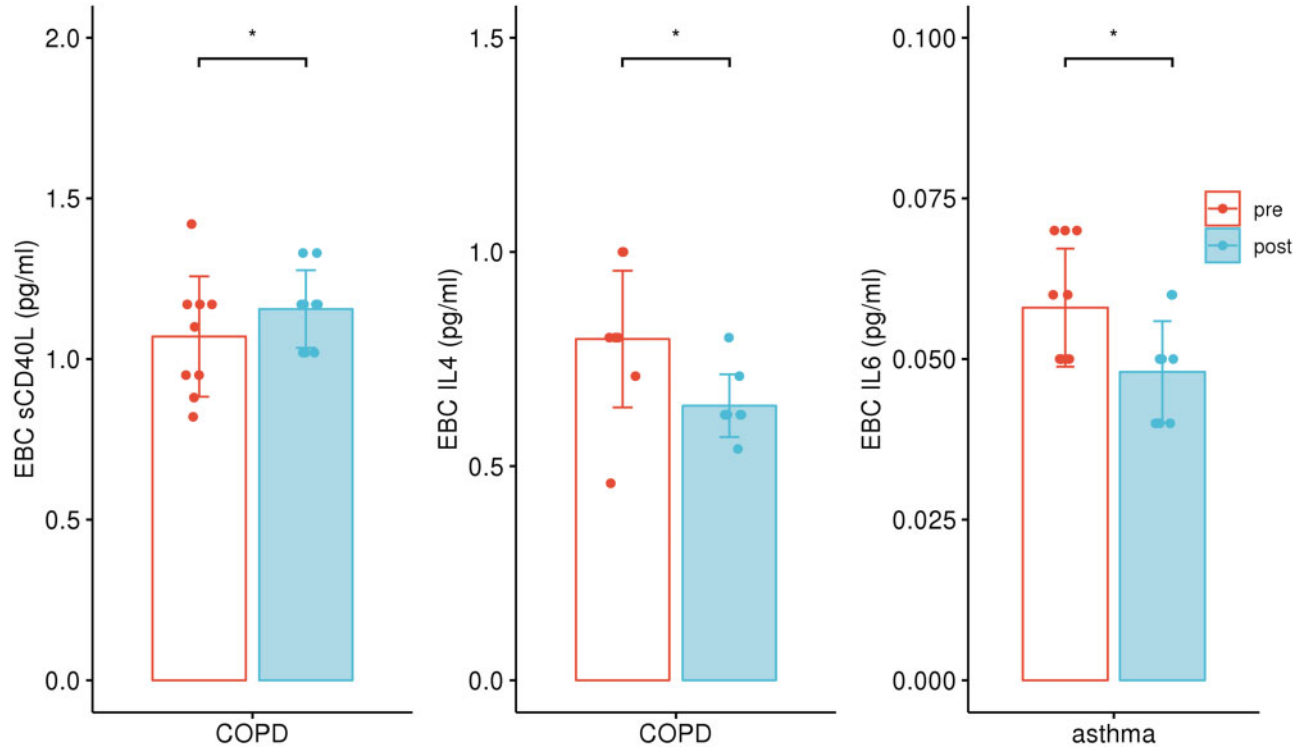

Figure 2. Cytokine levels in EBC before and after hydrogen inhalation. Soluble cluster of differentiation-40 ligand (sCD40L) level increased and IL-4 level decreased after hydrogen inhalation in COPD group. IL-6 level decreased after hydrogen inhalation in asthma group. *$P < 0.05$, paired t test.

Table 2. Proinflammatory mediators before and after hydrogen inhalation

		Before inhalation	After inhalation	*P* value
COPD				
Peripheral blood (pg/mL)	MCP1	564.70 + 164.67	451.51 + 166.39	0.019*
Exhaled breath condensate (pg/mL)	sCD40L	1.07 + 0.18	1.16 + 0.11	0.031*
	IL-4	0.80 + 0.15	0.64 + 0.07	0.025*
Asthma				
Peripheral blood (pg/mL)	MCP1	386.39 + 154.72	332.76 + 189.07	0.033*
	IL-8	5.25 + 2.10	4.49 + 2.79	0.023*
Exhaled breath condensate (pg/mL)	IL-6	0.06 + 0.01	0.05 + 0.01	0.007*

P values were obtained by paired samples *t* test after taking natural logarithms.
MCP1: monocyte chemoattractant protein-1; sCD40L: soluble cluster of differentiation-40 ligand; IL-4: interleukin-4; IL-8: interleukin-8; IL-6: interleukin-6.
*Values indicate significant differences ($P < 0.05$).

'XEN' inhalation, that there were some limitations. First, early study measured SOD activity, which calls for higher requirements for sample storage and measurement, not the content of SOD. Second, we did not investigate anti-oxidative properties of hydrogen, and previous studies evaluate oxidative damage by measuring the content of MDA and 8-hydroxydeoxyguanosine.[15,16]

As oxidants can promote inflammation by activating nuclear factor kappa-B (NF-kB) and other pathways, and oxidative stress can lead to a protease/antiprotease imbalance.[4] TNF-α and IL-1β are considered as initiator cytokines that initiate subsequent activations of other mediators, release of prostaglandin, and induction of chemotaxis of leukocytes due to inflammation.[17] The expression levels of TNF-α and IL-6 was proved to increase in the lungs with COPD,[18–20] and IL-6 may also contribute to acute lung injury as an inflammatory mediator,[21] and elevates in patients with acute respiratory distress syndrome (ARDS) or at risk of ARDS due to infection, injury or inflammatory diseases, which is associated with higher mortality.

Recent studies proved that 2% hydrogen can inhibit the expression of inflammatory cytokines, including IL-6 and mitigate lung injury through an antiapoptotic effect.[22,23] Qiu *et al.*[24] found 2% hydrogen could alleviate acute lung injury by reducing IL-6 and TNF-α expression. Liu *et al.*[25] proposed that hydrogen treatment might become a new and effective method for COPD and reported that 2% hydrogen inhalation significantly reduced the number of inflammatory cells in the bronchoalveolar lavage fluid (BALF) on a rat COPD model, and the mRNA and protein expression levels of TNF-α, IL-6, IL-17, IL-23, matrix metalloproteinase-12, caspase-3 and caspase-8, but increased the tissue inhibitor of metalloproteinase-1 expression. Furthermore, hydrogen inhalation ameliorated lung pathology, lung function and cardiovascular function and reduced the right ventricular hypertrophy index.[26] Terasaki *et al.*[27] found that

hydrogen-rich water reduced inflammatory cell infiltration of the lung, the expression of IL-6 in BALF and the accumulation of cells expressing MCP-1 and IL-6 in the airway with lung injury.

MCP-1, an activating factor of both monocytes and T lymphocytes, can act as a chemoattractant. Capelli *et al.*[28] reported increased concentrations of MCP-1 in BAL fluid from smokers with or without chronic bronchitis compared with healthy non-smoking subjects, and Traves *et al.*[29] found MCP-1 levels were significantly increased in sputum from COPD patients compared with non-smokers and healthy smokers, suggesting that this chemokine might play a role in the inflammatory cell recruitment associated with cigarette smoking. Fang *et al.*[30] found hydrogen inhalation inhibited the overexpression of MCP-1 in oxidant-induced endothelia and reduced inflammatory cells infiltration and proinflammatory cytokines (TNF-α, IL-6 and IL-8) production in cutaneous ischemia/reperfusion injury in a mouse model of pressure ulcer. Our study also found the MCP-1 level in peripheral blood significantly decreased after hydrogen inhalation, that hydrogen inhalation may produce protection against smoking.

Inflammatory cells, such as activated eosinophils and neutrophils identified in sputum and bronchial lavages in severe acute asthma are associated with increased levels of IL-5, IL-8[31] and IL-1β, IL-6 and TNF-α are detected in BAL from patients with symptomatic asthma and there is an increase in TNF-α production by macrophages after the late-phase response consecutive to allergen challenge,[32] while the immune inflammatory changes associated with COPD are linked to a tissue-repair and -remodeling process, that generates a broad spectrum of cytokines including TNF-α, TGF-β, IL-8 and so on.[33] To compare the effects of this hydrogen gas on COPD and asthma airway inflammation, previous studies based on COPD model animals showed hydrogen could alleviate the network of inflammatory factors like TNF-α, IL-6, IL-17 and IL-23, restore the balance of protease/antiprotease, and reduce apoptosis and alveolar structure damage[26]; while in asthmatic mice, the effect of hydrogen mainly focused on inhibition of NF-κB activation and activation of Nrf2 pathway, as well as to diminish TH_2 response.[14] In our study, IL-4 and IL-6 level in EBC decreased after inhalation in the COPD and asthma group, respectively; however, the values measured were near lower limit of detection and the results require more investigation.

Through the antioxidant pathway and the following anti-inflammatory effect, hydrogen may attenuate lung injury. Studies demonstrated hydrogen had a protective effect on hyperoxia-induced alveolar type II epithelial cell damage[34] and hydrogen inhalation could attenuate seawater instillation-induced acute lung injury in rabbits, that markedly improved lung endothelial permeability and decreased both MDA content and MPO activity in lung tissue. Hydrogen gas also alleviated histopathological changes and cell apoptosis, while Nrf2 and heme oxygenase 1 (HO-1) expressions were significantly activated and caspase-3 expression was inhibited.[11] Hydrogen-rich solution may also work that Terasaki *et al.*[35] found hydrogen therapy could attenuate irradiation-induced lung damage. Wang *et al.*[36] demonstrated hydrogen-rich saline alleviated lipopolysaccharide-induced acute lung injury by inhibiting excessive autophagy activation via the ROS/AMPK/mTOR pathway in mice with lung histopathological changes.

As previous studies on hydrogen inhalation were based on model animals or healthy human groups, this study provides important findings that even single inhalation of hydrogen was effective in modulating airway inflammation in patients with asthma and COPD. There are some limitations in this study. The first is the small sample size as this is a pilot study to evaluate the effect of hydrogen gas (XEN) inhalation on COPD and asthma, further studies are required to determine optimal dosing method for long-term treatment for patients. Other limitations include that we artificially set the time of hydrogen gas (XEN) inhalation to 45 min according to previous studies, that whether longer inhalation time may produce better effects remains unknown.

Conclusions

This study suggested that inhaled hydrogen (XEN) once with low concentration for 45 min had a positive therapeutic effect on airway inflammation in patients with asthma and COPD. Further studies are required in a larger sample size and longer duration of XEN treatments in asthma and COPD.

Funding

This work was supported by the National Key Research and Development Program of China (2018YFC1313600) and the National Key Basic Research Program of China (973 Program) (2015CB553402).

Conflict of interest. None declared.

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