Effects of inhaled β-caryophyllene on vascular stiffness in smokers: A randomized, double-blind, placebo-controlled trial

KAZUYA YAMADA[1], KOHEI TOYOTA[1], YAMATO TSUNODA[1], YOSHIHARU MATAHIRA[1], SHINICHI MATSUMURA[2], YURI YOSHIOKA[2], NOBUHIRO ZAIMA[3,4] and NAOKI UNNO[5,6]

[1]Sunsho Pharmaceutical Co., Ltd., Fuji, Shizuoka 419-0201; [2]Inabata Koryo, Co., Ltd., Osaka, Osaka 532-0027; [3]Department of Applied Biological Chemistry; [4]Agricultural Technology and Innovation Research Institute, Kindai University, Nara, Nara 631-8505; [5]Division of Vascular Surgery, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka 431-3192; [6]Division of Vascular Surgery, Hamamatsu Medical Center, Hamamatsu, Shizuoka 432-8580, Japan

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Abstract. Approximately 1.14 billion smokers worldwide are at risk of developing tumors, cardiovascular diseases and respiratory diseases. Smoking cessation is the first choice of health care; however, the disease should be attenuated in individuals who never stop smoking, which escalates medical costs. Therefore, alternative options are needed to manage the social burden. The present study proposed an alternative method to prevent such diseases by inhalation of β-caryophyllene (BCP). A placebo-targeted, dose-searching, double-blind, parallel-group comparative study was conducted on 19 subjects. The BCP intervention was performed using a flavor capsule inserted in a cigarette filter. The primary endpoint was the reducibility of brachial-ankle pulse wave velocity (baPWV). The secondary endpoints were confirmation of the bioavailability of BCP inhalation with cigarette smoke, confirmation of the effect of BCP inhalation on respiratory function, and association between respiratory function and blood concentration and baPWV reduction. The BCP concentration in the blood reached 4 ng/ml in the BCP 15% group 10 min after inhalation. The baPWV decreased in BCP-inhaling subjects whose initial baPWV was >1,300 cm/sec. The correlation analyses revealed that the higher the forced expiratory volume in 1 sec, the better the transition of baPWV. Inhaled BCP with cigarette smoke could reduce the baPWV and the risk of cardiovascular diseases in smokers. These findings indicated that with the introduction of BCP capsule-cigarettes in the future, smokers will be able to take care of their health, which may help reduce national medical costs. BCP microcapsules placed in cigarette wrapping paper may possibly reduce the risk of sidestream smoke and contribute to improved public health. This clinical research was retrospectively registered in the University Hospital Medical Information Network (UMIN)-Clinical Trials Registry with the following identifications: UMIN000048510 and UMIN000048512 on August 15, 2022.

Correspondence to: Professor Nobuhiro Zaima, Department of Applied Biological Chemistry, Kindai University, 3327-204 Nakamachi, Nara, Nara 631-8505, Japan
E-mail: zaima@nara.kindai.ac.jp

Abbreviations: %$FEV_{1.0}$, forced expiratory volume in 1 sec; %VC, % vital capacity; ABI, ankle brachial index; AUC, area under the curve of blood concentration; baPWV, brachial-ankle pulse wave velocity; BCP, β-caryophyllene; BCPO, β-caryophyllene oxide; CB2 receptor, cannabinoid receptor 2; COPD, chronic obstructive pulmonary disease; EPA, eicosapentaenoic acid; MMP, matrix metalloproteinases; MCT, medium-chain triglycerides; PPAR, peroxisome proliferator-activated receptors; PWV, pulse wave velocity

Key words: smoking/harm reduction, tobacco-related disease, BCP, baPWV, cardiovascular disease

Introduction

Smoking is a worldwide pastime, as shown by the existence of 1.14 billion current smokers in 2019 (1). The report mentioned that both the ratio and population of smokers were decreasing; for example, the percentage decreases from 1990 to 2019 were 37.7% for women and 27.5% for men. Although the smoking population is declining, the number of people who are dying from disease caused by smoking is increasing. From 2011 to 2019, the number of deaths due to smoking increased from 5.40 to 7.69 million people worldwide (2). Health hazards from smoking include malignant tumors, cardiovascular diseases, and respiratory diseases. In 2010, the number of deaths was reported as 77,400 due to malignant tumors, 18,100 due to cardiovascular disease, and 33,400 due to respiratory disease (3). These diseases caused by smoking contribute to rising medical costs worldwide; therefore, the health of smokers is also important to consider in government health policies.

Examples of cardiovascular diseases caused by smoking include ischemic heart disease, stroke, and aortic aneurysm and dissection. One of the most important risk factors for these diseases is the hardening of blood vessel walls, which is caused by nicotine inhalation (4). The pulse wave velocity (PWV) is one of the indicators of arterial wall sclerosis (5).

There are several PWV measurement methods; however, in recent years, brachial-ankle PWV (baPWV), which is easy to measure, has been widely used (6). Since baPWV shows a correlation with the Framingham risk score (7,8), it may be considered an index that reflects the total cardiovascular risk. For example, a baPWV of 1400 cm/sec corresponds to a moderate Framingham risk score (8) and increases the risk of developing hypertension (9). A meta-analysis also showed that baPWV is an independent predictor of prognosis, with a 12% increase in cardiovascular disease incidence with an increase of 100 cm/sec (10).

These diseases caused by smoking have been reduced mainly through policy changes that induce smoking cessation. Moreover, some cigarette manufacturers have launched risk-reducing products such as heat-not-burn tobacco. However, these correspondences had limitations because there are hundreds of millions of people who want to quit smoking, but cannot. Therefore, alternative options are needed to manage their health. Some statins and food ingredients such as eicosapentaenoic acid (EPA) decrease baPWV (11-13), which may be useful for improving smokers' health. We aimed to develop a method that can be easily ingested with safer food ingredients instead of pharmaceuticals as an alternative option for smokers. To overcome these problems, we focused on inhaling volatile components from cigarette smoke using a flavor capsule (14). The volatile food additive that we focused on is β-caryophyllene (BCP), because BCP is a compound that reduces baPWV and known as an agonist of cannabinoid receptor 2 (CB2 receptor) (15) with anti-inflammatory effects. Vascular walls are destroyed and hardened by matrix metalloproteinase (MMP) production via inflammation caused by nicotine ingestion (16,17). Inhaled BCP is transferred into the blood and can prevent vascular fiber degradation and blood vessel hardening in mice (18,19). Vascular fibers play a role in maintaining the elasticity and strength of the vessels; therefore, the prevention of vascular fiber degradation by BCP is directly linked to reduced baPWV. Based on these results, we hypothesized that BCP suppresses the hardening of the blood vessel wall, resulting in a decrease in baPWV.

In terms of palatability, BCP has a weak waxy or woody odor, so the odor is drowned out by the smell of cigarette smoke. BCP is present in essential oils at concentrations of 20% for clove bud oil, 20% for basil oil, 16% for oregano oil, 15% for hop oil, 11% for cinnamon oil, 8% for rosemary oil, 7% for black pepper oil, and 5% for lavender oil (20,21). A clinical study related to BCP has also been reported (22).

Herein, we aimed to examine if inhaling BCP with cigarette smoke reduced the baPWV of healthy smokers.

Materials and methods

Design of the research. All recruited participants were healthy smokers; the intervention was inhaling BCP with cigarette smoke, and the intervention group was compared against the initial status and a placebo group. The outcomes were changes in blood BCP concentration over time and the effect of reducing baPWV.

The blood levels of BCP and BCP oxide (BCPO) were measured in this single-dose study. Vascular function was measured in a placebo-targeted dose-searching, double-blind, parallel-group comparative study.

Ethical review. After deliberation and approval by the Institutional Review Board of Sunsho Pharmaceutical Co., Ltd. (approval number: 21001 for vascular function, and 21002 for BCP concentration in blood), this study was carried out in accordance with the Declaration of Helsinki and with ethical considerations. In addition, prior to the start of the study, informed consent was requested from each subject individually, and the test was conducted after explaining that participation in this test was voluntary and that there was no disadvantage, even if consent was not given. Written informed consent was obtained from all participants in this study.

Subjects. A questionnaire was administered at the time of recruitment, and a screening survey was conducted targeting those who met the self-report entry criteria and did not violate the exclusion criteria. Healthy subjects who met the screening criteria and were judged by us to be appropriate for participation in this study were selected as the subjects of this study. In the course of the screening test, if it became clear that the test results were inconsistent with the self-reported contents, they were excluded from the analysis, unless there was a specific reason. In addition, if the instruction in the subject management items was violated during the research period, or if there was a big problem in the reliability of the data due to troubles during the research period, it was considered appropriate to treat them as dropouts or exclude them from the analysis target.

The eligibility criteria were as follows: healthy adult men and women and smokers who smoked regular cigarettes. The exclusion criteria were as follows: non-smokers, smokers who smoked tobacco other than cigarettes (pipes), smokers who smoked non-regular type (slim type) cigarettes, those who planned to quit smoking during the experimental period, those who were being treated for disease or who were planning to receive treatment, those who hoped to become pregnant during the experimental period, those who were pregnant or lactating, those who were taking medication, or those who consumed health foods (excluding products whose active ingredients were only vitamins and minerals) on a daily basis. After the initial survey, those who met the exclusion criteria were excluded. No other screenings were performed.

Intervention and inspection methods. BCP (Relaxphytone®) was purchased from Inabata Koryo Co. Ltd. (Osaka, Japan). Medium-chain triglycerides (MCT) were purchased from KAO (Tokyo, Japan). The encapsulation of the BCP/MCT solution was conducted by Sunsho Pharmaceutical Co., Ltd. (Shizuoka, Japan) using the dropping method. The diameter of the capsule was 3.4 mm and the weight of the core was set to 19.30 mg. Three types of capsules were manufactured and their compositions are described below.

Capsule 1: BCP 5.79 mg, MCT 13.51 mg (BCP 30%)
Capsule 2: BCP 2.90 mg, MCT 16.40 mg (BCP 15%)
Capsule 3: MCT 19.30 mg (placebo)

The cigarettes that the subjects normally smoked were inquired through the initial questionnaire survey. The capsules were inserted into the cigarette filters corresponding to the assigned group. BCP was inhaled via smoking after insertion.

For the blood levels of BCP and nicotine measured in the single-dose study, eight volunteer subjects were assigned as follows: four subjects for capsule 1, three subjects for capsule 2, and one subject for capsule 3. The allocation method was the same as that used in the vascular function study.

Blood (5 ml) was collected six times before smoking and 10, 20, 40, 90 and 180 min after smoking. After blood collection, plasma was collected by centrifugation. Blood plasma samples were frozen and stored at -80˚C until analysis.

BCP and BCPO concentrations in the serum were analyzed using gas chromatography-mass spectrometry (Agilent 7890B-5977B MSD; Agilent Technologies, Santa Clara, CA, USA), a thermal desorption unit, programmable temperature vaporization inlet, and multipurpose sampler with dynamic head space option (all Gerstel GmbH & Co.KG, Mülheim an der Ruhr, Germany) 39. MSD ChemStation v.F.01.03. 2357 (Agilent), and Mass Hunter v.B.07.05.2479 (Agilent) were used for data analysis. Tenax TA cartridges were released from Tenax TA traps using a thermal desorption cold-trap setup (thermal desorption spectrometer; Markes International, Ltd., Llantrisant, RCT, UK). An InertCap WAX column (length: 60 m, outer diameter: 0.25 μm, I.D.: 0.25 mm, GL Sciences, Inc., Tokyo, Japan) was used for component separation. The oven temperature was programmed as follows: initial temperature, 40˚C; ramp rate, 3˚C/min (40 to 145˚C) and 10˚C/min (145 to 240˚C); final temperature, 240˚C for 5 min. The He inlet pressure was controlled using an electronic pressure control system to achieve a constant column flow rate of 1 ml/min. Mass spectrometry analysis was performed in the ionization mode at a voltage of 70 eV.

For the vascular function measured in the placebo-targeted study, 19 volunteer subjects were assigned as follows: seven subjects for capsule 1, seven subjects for capsule 2, and five subjects for capsule 3.

The test period was 12 weeks from September to December 2021, and the total number of observation days was four: before, 4 weeks after, 8 weeks after, and 12 weeks after the start of the test period.

Cigarettes with a capsule in the center of the filter that the subjects normally smoked were provided. Capsules were crushed immediately before smoking. The number of cigarettes smoked per day was counted by collecting cigarette butts. Butts were also collected for two weeks before the monitoring period.

During the observation period, blood pressure, baPWV, and ankle brachial index (ABI) were measured using a BP-203PRE II (Fukuda Colin Co., Ltd., Tokyo, Japan). The % vital capacity (%VC) and forced expiratory volume in 1 sec ($\%FEV_{1.0}$) were measured using Chestgragh HI-10 (Chest Co., Ltd., Tokyo, Japan).

Allocation and blinding method. Subjects were randomly assigned so that sex differences, age, daily number of cigarettes, smoking history, amount of nicotine and tar in cigarettes, and care about dietary balance were as similar as possible among the groups.

The secretariat, who was not in charge of measurement and analysis, managed and locked the correspondence table of the subject name, subject number for analysis, and capsule. The lock was unlocked after all data collection was completed. The assigned group or measurement results were not disclosed to the subjects.

Definition of endpoints. The primary endpoint of this study was the reduction in baPWV by inhaling BCP via cigarette smoke. The secondary endpoints were investigation of the bioavailability of inhaled BCP with cigarette smoke; confirmation of the effect of BCP inhalation on respiratory function such as %VC and $\%FEV_{1.0}$; and association between respiratory function and blood concentration and baPWV reduction.

Statistical considerations. All statistical calculations were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria, version 4.0.3). More precisely, it is a modified version of the R commander (version 2.7-1) that was designed to add statistical functions frequently used in biostatistics (23). The Friedman test was used to compare time-series data within the group for vascular and respiratory function. The Mann-Whitney U test was used for comparisons between the two groups, and the Steel-Dwass multiple comparison method following the Kruskal-Wallis test was used for comparisons among the three groups. The Spearman's rank correlation test was used to determine the correlation between the two parameters. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Outline of research. An outline of this study is presented in Fig. S1. After enrolment and gathering of informed consent from 38 people, 19 people were selected based on the inclusion and exclusion criteria. These participants were randomly assigned to three groups, five people for placebo group, seven people for BCP 15% capsule group, and seven people for BCP 30% capsule group. Then, the BCP was placed within their cigarette capsules (Fig. 1). The measurements were performed four times and the data were collected and analyzed. After the end of the intervention, some subjects, one person for placebo group, three people for BCP 15% capsule group, and four people for BCP 30% capsule group, underwent a blood sampling test.

Baseline characteristics. The baseline characteristics of the groups are shown in Table I. There were no statistically significant differences in age, cigarettes per day, nicotine per day, tar per day, pack year, initial baPWV, initial %VC, and initial $\%FEV_{1.0}$ among the three groups. The demographic characteristics of the groups, other than the amount of BCP intake, were comparable.

BCP and BCPO concentration in blood. The results are summarized in Fig. 2. The maximum concentration of BCP reached 4.2 ng/ml 10 min after smoking in the BCP 15% group. Subsequently, the concentration decreased exponentially. In contrast, the maximum concentration reached 2.6 ng/ml 90 min after smoking in the BCP 30% group. The BCP concentration

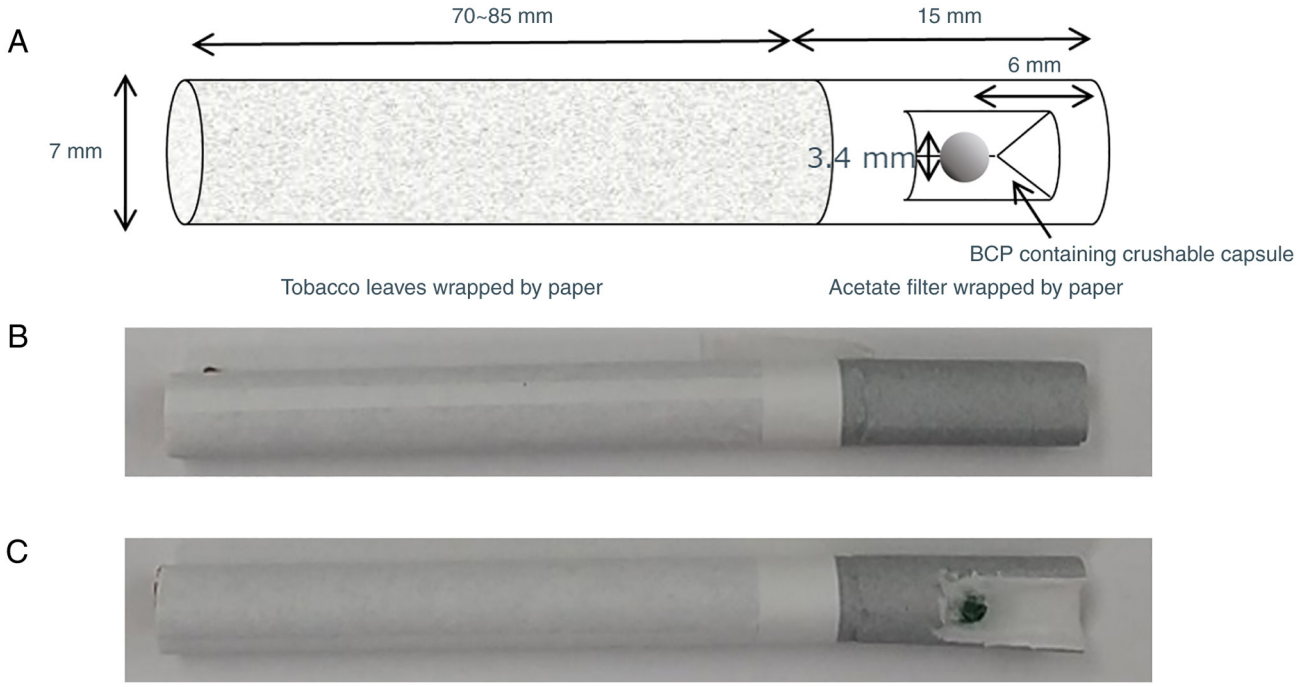

Figure 1. Capsule-inserted cigarette sample used in this study. (A) Schematic diagram and dimension of the capsule cigarette. (B) An example of a cigarette with a capsule in the filter. (C) A cigarette with a filter that exposes the capsule with a slit. BCP, β-caryophyllene.

in the placebo group was constant at 1 ng/ml. The concentration of BCPO was similar to that of BCP.

Vascular function. The baPWV transition of the BCP and placebo groups is shown in Fig. 3A for subjects whose initial baPWV was >1,300 cm/sec. In the BCP group, baPWV was reduced at 4, 8 and 12 weeks after the intervention started, and the difference from the initial value was statistically significant (P=0.03, 0.09 and 0.03). In contrast, the baPWV did not change significantly in the placebo group. The difference in baPWV between the BCP and placebo groups for four weeks was statistically significant (P=0.08). In all cases, there was a small difference between the BCP and placebo groups, but this was not statistically significant (Fig. S2A).

There was little difference among the BCP 30%, BCP 15%, and placebo groups for all cases. However, the baPWV transitions of the two BCP intervention groups were less than those of the placebo group at 4, 8 and 12 weeks after intervention for the sub-analysis of subjects whose initial baPWV was larger than 1300 cm/sec (Fig. S2B and C).

Except for baPWV, vascular function data such as diastolic and systolic blood pressure (mean of left and right arms), pulse pressure, heart rate, and ABI were not significantly changed during the 12 week study (Fig. S3A).

Respiratory function. The transition of %VC and $\%FEV_{1.0}$ for each group is shown in Fig. S4. Both %VC and $\%FEV_{1.0}$ did not change significantly in any of the groups.

Correlation analysis. The correlation between $\%FEV_{1.0}$ and baPWV transition for 12 weeks was strong and statistically significant (Fig. 3B). The correlations between $\%FEV_{1.0}$ and the area under the curve of blood concentration (AUC) of BCP and between the AUC of BCP and baPWV transition at 12 weeks are shown in Fig. S5. These correlations were moderate but not statistically significant.

Number of cigarettes smoked. The number of cigarettes smoked by each participant was monitored during the study and is plotted in Fig. S6. There was no correlation between the number of cigarettes smoked and the amount of BCP in capsules.

Discussion

In this study, we determined the bioavailability of inhaled BCP and its effects on vascular stiffness by inhaling BCP via smoking. As a result of the bioavailability, inhaled BCP was transferred to the blood and reached 4 ng/ml in serum. The baPWV decreased by 10% in subjects whose initial baPWV was >1,300 cm/sec, but BCP did not have any effect on respiratory function such as %VC and $\%FEV_{1.0}$.

It has been reported that the bioavailability and absorption rate of inhaled compounds are higher than those of oral compounds (24). In the case of cigarette smoking, it was reported that the maximum blood concentration of nicotine was 10-20 ng/ml, and the time for maximum blood concentration was approximately 10 min (25-27). For BCP, the results of this study were consistent with these reports regarding the maximum blood concentration and the transition of blood concentration. BCP was detected in the blood of the placebo group. The reason for this detection was considered to be BCP ingested from food. It is known that 0.4-0.5 mg per day per person of BCP is ingested from food in Europe and the United States (28).

In this study, baPWV was reduced by 10% in the BCP group when the initial baPWV was larger than 13,00 cm/sec. The range of initial baPWV was 1300-1600 cm/sec; hence,

Table I. Baseline characteristics of subjects in each group presented as mean (maximum, and minimum).

Characteristic	Placebo	BCP 15%	BCP 30%	P-value
Number	5	7	7	
Age, years	36 (22, 58)	32 (31, 55)	35 (20, 49)	0.782
BCP (mg)/day	0.0 (0.0, 0.0)	27.8 (12.2, 46.1)	44.6 (30.7, 95.5)	0.002
Cigarettes/day	8.9 (4.9, 28.7)	9.60 (4.2, 15.9)	7.7 (5.3, 16.5)	0.915
Nicotine (mg)/day	11.1 (0.5, 20.7)	4.8 (0.7, 20.1)	3.3 (1.0, 25.2)	0.782
Tar (mg)/day	129.0 (5.0, 236.1)	57.6 (7.3, 234.3)	40.8 (10.2, 201.8)	0.687
Pack year	9.50 (0.49, 26.10)	8.03 (2.52, 27.82)	5.00 (0.00, 14.03)	0.51
Initial PWV (cm/sec)	1,368.5 (1,118.0, 1,546.0)	1,472.50 (1,166.0, 1,632.0)	1,507.50 (1,032.5, 1,602.0)	0.617
Initial %VC	118 (99, 149)	102 (79, 129)	99 (76, 113)	0.156
Initial $\%FEV_{1.0}$	83.0 (76.5, 91.4)	83.7 (57.4, 89.7)	75.4 (19.0, 100.0)	0.788

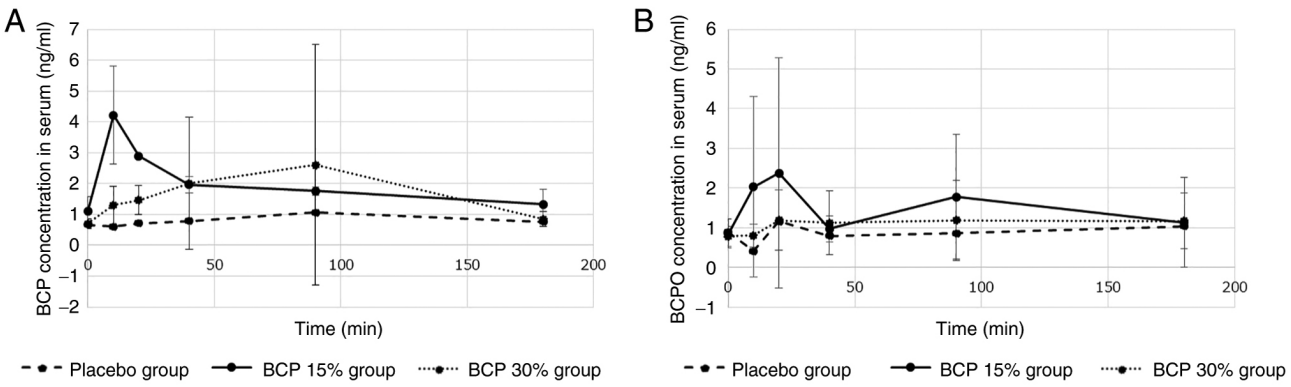

Figure 2. Time series of the concentrations of BCP and BCPO in blood after smoking. Time series of the concentrations of (A) BCP and (B) BCPO in blood after smoking. Dotted lines show the BCP 30% group, solid lines show the BCP 15% group, and dashed lines show the placebo group. BCP, β-caryophyllene; BCPO, β-caryophyllene oxide.

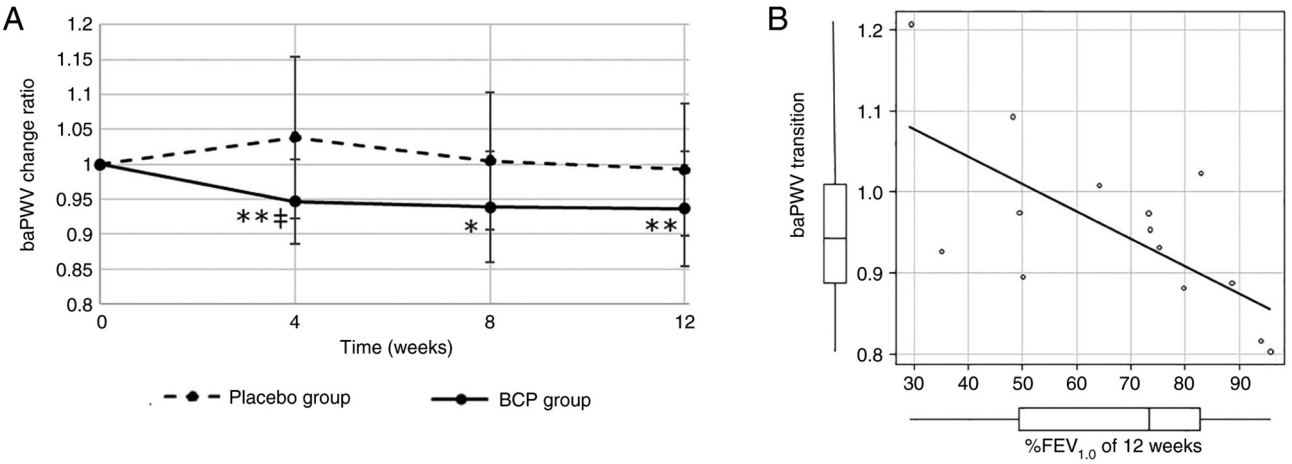

Figure 3. baPWV transition and the correlation between baPWV transition and $\%FEV_{1.0}$ over 12 weeks. (A) The baPWV transition in the BCP and placebo groups during this study for subjects whose initial baPWV was >1,300 cm/sec. Solid lines represent the BCP group, and dashed lines indicate the placebo group. ‡P<0.1 vs. the placebo; **P<0.05 vs. the initial baPWV; *P<0.1 vs. the initial baPWV. (B) Correlation between $\%FEV_{1.0}$ in 12 weeks and baPWV transition. Spearman's rank correlation coefficient is -0.666, and the P-value is 0.0115. BCP, β-caryophyllene; baPWV, brachial-ankle pulse wave velocity; $\%FEV_{1.0}$, forced expiratory volume in 1 sec.

10% of baPWV means 130-160 cm/sec. In previous reports, the one-year intervention of EPA (1,800 mg/day) reduced baPWV, and the changes in baPWV of the EPA and placebo groups were -10 and +40, respectively (13). In the case of medicines for treating dyslipidemia, ezetimibe and simvastatin reduced the aortic PWV by 10% for six weeks (11). Other reports showed that a one-year intervention with fluvastatin also reduced baPWV from 1,800 to 1,650 cm/sec (12). Compared to these

functional foods and medicines, BCP was considered to have the same or greater effect in reducing baPWV. It is known that the baPWV is influenced by blood pressure. However, in this study, baPWV was reduced, but blood pressure did not change. This result suggests that blood vessel flexibility is restored without lowering the blood pressure.

There was no difference in %VC and $\%FEV_{1.0}$ during the 12 week monitoring period comparing the BCP capsule and placebo groups. We concluded that respiratory function did not change significantly during the study; therefore, the BCP did not adversely affect respiratory function. Participants with low $\%FEV_{1.0}$ could not capture BCP efficiently in the blood through the lungs. People with a low $\%FEV_{1.0}$ are those suffering from chronic obstructive pulmonary disease (COPD), and it is thought that their airway is narrow and gas cannot be inhaled well because the air in the alveoli does not change. In fact, it was reported that patients with COPD and low $\%FEV_{1.0}$ had high blood $PaCO_2$ and low PaO_2 (29). People with a low $\%FEV_{1.0}$ are considered to have difficulty in taking BCP, as well as oxygen and carbon dioxide. In this study, people with a low $\%FEV_{1.0}$ had low blood concentrations of BCP and a low baPWV reducing transition. Individual differences of $\%FEV_{1.0}$ and/or absorption efficiency of BCP may have affected the blood concentration of BCP and vascular function.

The number of cigarettes smoked was not affected by BCP, suggesting that the improvement effect of BCP is not because of the reduction of nicotine intake but the activity of BCP itself. BCP is reported to be an agonist of the CB2 receptor (15). Compared to the cannabinoid type 1 receptor, which is expressed in central nervous cells and is related to mental effects, the CB2 receptor is expressed mainly in immune cells and is related to inflammation. BCP has also been reported to interact with peroxisome proliferator-activated receptors (PPARs), in particular PPAR α and γ (30-32). PPARs are involved in both metabolic and inflammatory responses. According to some reports, BCP shows anti-inflammatory and anti-oxidative stress (33-37). Previous studies have reported that nicotine induces aortic stiffness with degeneration of the aorta in mice (4,16,38). We recently reported that inhalation of BCP attenuates nicotine-induced murine aortic stiffness via the CB2 dependent pathway (19). These studies suggest that BCP can reduce inflammation in the human body caused by hazardous substances such as aldehydes in the mainstream smoke of cigarettes.

A limitation of this study was the sample size and monitoring period. In this study, only a few statistically significant differences in baPWV reduction were observed owing to the small number of subjects. To confirm the preventive effect of BCP, it is necessary to have deteriorated vascular and respiratory functions in the placebo group, which would take at least one year. Moreover, this study contains content that makes use of patents that are owned by Sunsho Pharmaceutical Co., Ltd., INABATA KORYO, Co., Ltd., and Kindai University.

In conclusion, BCP with cigarette smoke can be inhaled into the blood, thereby reducing the baPWV in humans. BCP is, to the best of our knowledge, the first compound that can comprehensively contribute to the health of smokers who cannot quit smoking and can be easily ingested. BCP microcapsules can be placed in cigarette wrapping paper to reduce the risk of sidestream smoke and contribute to improved public health.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

KY conceptualized and designed the details of this research, collected clinical data and was a major contributor in writing the manuscript. KT and YT analyzed data statistically, collected clinical data, and reviewed and edited the manuscript. YM designed the details of this research, and reviewed and edited the manuscript. SM and YY analyzed serum samples by gas chromatography-mass spectrometry, provided raw materials, and reviewed and edited the manuscript. NZ and NU conceptualized and designed the details of this research, and reviewed and edited the manuscript. KY, SM, YY, NZ and NU confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Ethics approval was obtained from the Institutional Review Board of Sunsho Pharmaceutical Co., Ltd. (approval no. 21001 for vascular function, and 21002 for BCP concentration in blood). Written informed consent was given by each subject individually.

Patient consent for publication

Written informed consent was obtained from the participants in this study for publication of this paper.

Competing interests

KY, KT, YT, and YM are employees of Sunsho Pharmaceutical Co., Ltd., and SM and YY are employees of INABATA

KORYO, Co., Ltd. Kindai University, INABATA KORYO, Co., Ltd., and Sunsho Pharmaceutical Co., Ltd. applied patents related to this research (PCT/JP2021/040234, JP/2021/149802, JP/2021/149803, JP/2022/074711). BCP used in this research was purchased from INABATA KORYO, Co., Ltd. The BCP-containing capsules and placebo capsules were manufactured by Sunsho Pharmaceutical Co., Ltd.

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