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Evidence of Improvements to Arterial Stiffness Among Regular Users of Combustible Cigarettes – Effect of Inhalation of β-Caryophyllene: A Randomized, Double-Blind, Placebo-Controlled Study –*

by

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SUMMARY

Nicotine consumption is known to be a risk factor for cardiovascular disease. β-Caryophyllene (BCP), a sesquiterpene with anti-inflammatory properties, was investigated in a randomized, double-blind, placebo-controlled trial to see if smoking cigarettes with BCP-containing capsules could improve aortic stiffness. In this study, 84 adult smokers were randomly assigned to either a BCP group or a placebo group. They smoked capsule-loaded cigarettes for 12 weeks, and various health parameters were measured every 4 weeks. The primary focus was on changes in brachial-ankle pulse wave velocity (baPWV), which measures arterial stiffness. The results showed that blood BCP levels increased only in the BCP group, while nicotine levels rose in both groups. For participants with a high baseline baPWV (≥ 1,400 cm/s), significant reductions in baPWV were observed in the BCP group at weeks 4 and 8. Additionally, baPWV at week 4 was significantly lower in the BCP group compared to the placebo group. No adverse effects were reported. In conclusion, smoking BCP-containing cigarettes improved arterial stiffness in participants with high baseline baPWV without causing any adverse effects. [Contrib. Tob. Nicotine Res. 34 (2025) 107–116]

KEYWORDS

Smoking/harm reduction; tobacco-related disease; β-caryophyllene; brachial-ankle pulse wave velocity; cardiovascular disease.

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ZUSAMMENFASSUNG

Es ist bekannt, dass Nikotinkonsum ein Risikofaktor für Herz-Kreislauf-Erkrankungen ist. β-Caryophyllen (BCP), ein Sesquiterpen mit entzündungshemmenden Eigenschaften, wurde in einer randomisierten, doppelblinden, placebokontrollierten Studie eingesetzt, um festzustellen, ob das Rauchen von Zigaretten mit BCP-haltigen Kapseln die Aortenverhärtung verbessern kann. In dieser Studie wurden 84 erwachsene Raucher nach dem Zufallsprinzip entweder einer BCP-Gruppe oder einer Placebo-Gruppe zugeteilt. Sie rauchten 12 Wochen lang Zigaretten, denen BCP-haltige Kapseln zugesetzt wurden, und alle 4 Wochen wurden verschiedene Gesundheitsparameter gemessen. Das Hauptaugenmerk lag auf den Veränderungen der Pulswellengeschwindigkeit zwischen Oberschenkel und Knöchel (baPWV), die die arterielle Steifigkeit misst. Die Ergebnisse zeigten, dass der BCP-Gehalt im Blut nur in der BCP-Gruppe anstieg, während die Nikotinwerte in beiden Gruppen zunahmen. Bei Teilnehmern mit einem hohen baPWV-Ausgangswert (≥ 1.400 cm/s) wurde in der BCP-Gruppe in Woche 4 und 8 eine signifikante Verringerung des baPWV beobachtet. Außerdem war der baPWV in Woche 4 in der BCP-Gruppe signifikant niedriger als in der Placebo-Gruppe. Es wurden keine unerwünschten Wirkungen berichtet. Zusammenfassend lässt sich sagen, dass das Rauchen von BCP-haltigen Zigaretten die arterielle Steifigkeit bei Teil-nehmern mit hohem Ausgangs-BaPWV verbesserte, ohne dass es zu unerwünschten Wirkungen kam. [Contrib. Tob. Nicotine Res. 34 (2025) 107–116]

RESUME

La consommation de nicotine est connue pour être un facteur de risque de maladie cardiovasculaire. Le β-caryophyllène (BCP), un sesquiterpène aux propriétés anti-inflammatoires, a fait l'objet d'une étude randomisée, en double aveugle, contrôlée par placebo, afin de déterminer si le fait de fumer des cigarettes avec des capsules contenant du BCP pouvait améliorer la rigidité aortique. Dans cette étude, 84 fumeurs adultes ont été répartis au hasard entre un groupe BCP et un groupe placebo. Ils ont fumé des cigarettes chargées de capsules pendant 12 semaines et divers paramètres de santé ont été mesurés toutes les 4 semaines. L'accent a été mis sur l'évolution de la vitesse d'onde de pouls brachial-cheville (baPWV), qui mesure la rigidité artérielle. Les résultats ont montré que les niveaux de BCP dans le sang n'ont augmenté que dans le groupe BCP, tandis que les niveaux de nicotine ont augmenté dans les deux groupes. Pour les participants ayant un baPWV élevé au départ (≥ 1400 cm/s), des réductions significatives du baPWV ont été observées dans le groupe BCP aux semaines 4 et 8. De plus, le baPWV à la semaine 4 était significativement plus bas dans le groupe BCP que dans le groupe placebo. Aucun effet indésirable n'a été signalé. En conclusion, le fait de fumer des cigarettes contenant du BCP a amélioré la rigidité artérielle chez les participants ayant un baPWV élevé au départ, sans provoquer d'effets indésirables. [Contrib. Tob. Nicotine Res. 34 (2025) 107–116]

1. INTRODUCTION

Cardiovascular disease has the highest mortality rate worldwide (1), and nicotine intake is a risk factor for aortic degeneration (2). Despite the known risks and awareness, there are ~1.1 billion smokers worldwide (3). When a smoker shows signs of cardiovascular disease, doctors advise on smoking cessation. Encouraging smokers to quit smoking and achieving smoking cessation is the best approach, but it is challenging to ensure compliance among all individuals. Therefore, this study aimed to investigate the effectiveness of a secondary strategy by adding a flavoring substance with anti-arteriosclerotic properties to cigarette filters, allowing smokers to smoke while potentially mitigating the adverse cardiovascular effects of nicotine. Until now, using a similar approach, studies have been reported examining the effects of supplement interventions for smokers (4) and exercise interventions for smokers (5). Attaching flavored capsules to cigarette filters is a common form of smoking (6).

β-Caryophyllene (BCP), a naturally occurring sesquiterpene found in the essential oils of clove (7, 8), black pepper, and other spices, is used as a flavoring agent in food. Its ingestion rate differs by country, with 0.508 mg/day and 0.389 mg/day of BCP ingested in the United States and Europe, respectively (9). Additionally, BCP binds to CB2 receptors (10) and PPARγ (11), exerting anti-inflammatory (12) and lipid-metabolism-improving effects (13). For tobacco related diseases, essential oils containing BCP have been reported to have a positive effect on COPD (14). BCP also has antimutagenic properties (15) and its anticancer effects (16) have been reviewed. Specifically, its favorable effect on miR-659-3p-targeted sphingosine kinase 1 in lung cancer cells has been reported (17). In our previous studies, inhaled BCP was detected in the blood and aortic tissues of mice (18, 19). Therefore, we subsequently reported that BCP inhalation significantly inhibited the histological loss of elastic fibers and alleviated the increased stiffness of blood vessels in a mouse model of nicotine-induced loss of vascular elasticity (19). In addition to these animal studies, an exploratory clinical study with a small number of participants confirmed that BCP can be detected in human blood after smoking a single cigarette with a BCP-containing capsule. Moreover, a randomized, double-blind, placebo-controlled study measuring brachial-ankle pulse wave velocity (baPWV) as the endpoint after 12 weeks of using BCP-containing capsules confirmed their safety and showed a trend toward improvement in baPWV (20). The baPWV is an index that measures the stiffness of blood vessels, and its clinical significance has been demonstrated, with a 20% increase in baPWV being associated with a 1.3-fold increase in cerebrocardiovascular disease (21). A 100-cm/s increase is linked to a 12% increase in cardiovascular disease (22).

Therefore, we hypothesized that adding BCP, a volatile food ingredient, to such capsules and inhaling BCP with cigarette smoke would positively affect sclerotic arterial vessels with increased baPWV. Consequently, we recruited a larger number of healthy volunteer smokers and randomly assigned them to the BCP or placebo groups to prospectively evaluate the benefits and safety of using BCP-containing capsules for 12 weeks.

2. METHODS

2.1 Trial design

This was a randomized, double-blind, parallel-group study with a 1:1 allocation ratio between the BCP and placebo groups. The primary endpoint was the difference in baPWV change at 12 weeks between the BCP with placebo. Secondary endpoints included the safety of BCP capsules, among other items (Table S1).

2.2 Study population

The recruitment process (November 2, 2022, to May 29, 2023) was outsourced to a specialized company to enroll Japanese residents based on the criteria listed in Table S2. An additional table lists eligible cigarette brands whose tobacco was confirmed to not contain BCP (Table S3). After obtaining informed consent, screening tests (December 10, 2022, to May 29, 2023) (Table S4) were conducted at the Three-One Clinic and Watanabe Hospital in Tokyo, Japan. Participants who met the inclusion (Table S5) but not the exclusion (Table S6) criteria were enrolled from December 10, 2022, and followed up from March 4, 2022, to September 30, 2023. The inclusion and exclusion criteria, except for baPWV, were designed to select healthy volunteers. The lower limit of baPWV, 1,300 cm/s, was set based on the results of previous studies (20) to select subjects with moderately hardened arteries. Discontinuation criteria were also established (Table S7).

2.3 Randomization

For eligible participants, a drug number that did not contain information regarding the treatment group was issued on the registration system built on an electronic data capture system, and the allocator was notified. Allocation to groups was performed using a stratified substitution block method with a BCP:Placebo ratio of 1:1 and a random block size of 2 or 4. Two allocation factors were used:

- baPWV < 1,500 cm/s *vs.* ≥ 1,500 cm/s and
- forced expiratory volume in 1 s (%FEV1.0) < 80% *vs.* ≥ 80%.

The allocation staff assigned drug numbers to capsules according to an allocation table that was inaccessible to the principal investigator and study participants. The allocation was outsourced to Stat Academy LLC (Kobe, Japan) and incorporated by the CMIC HealthCare Institute Co., Ltd. (Tokyo, Japan).

2.4 Blinding

After the assignment, the participants and intervention providers remained blinded by presenting only randomized codes. These codes were managed electronically on electronic data capture and kept strictly confidential. At the end of the study, the principal investigator asked Satt Co., Ltd. (Tokyo, Japan), the institution in charge of data management, to open key and output codes.

2.5 Sample preparation

BCP was supplied by Inabata Koryo Co., Ltd. (Osaka, Japan). Medium-chain triglycerides (MCT) were purchased from KAO (Tokyo, Japan). The capsules (3.4 mm diameter) were prepared by Sunsho Pharmaceutical Co., Ltd. (Fuji, Japan) using the dropping method. The liquid compositions for the placebo and BCP capsules were 19.30 mg MCT and 2.90 mg BCP with 16.40 mg MCT (15% BCP), respectively. The BCP amount per capsule was determined as described in Supplementary method 1.

2.6 Intervention and inspection methods

Four weeks before the initiation date (day 0), both the capsule-attaching device and capsules (Figure S1) were provided to the participants, along with a written manual, during a visit to the study site. After breaking the capsule in the filter prior to smoking, participants in both groups smoked one cigarette with a capsule attached to a facility adjacent to the research institute, as described previously (20). Blood samples were collected in the research facility four times (5 mL each): before smoking and 10, 20, and 40 min after starting smoking. Blood plasma was obtained by centrifugation, frozen, and stored at −80 °C until analysis. BCP and nicotine in the blood were quantified as described in the Supplementary method 2.

Participants were asked to visit the clinic on the initiation date (day 0) and at weeks 4, 8, and 12. During each visit, including day 0, the tests listed in Table S8 were performed, including the original questionnaire survey (Table S9). In contrast, the SF-36 questionnaire (40, 41) was administered only on day 0 and week 12. Capsules and capsule-attaching devices were again provided to the participants in both groups on day 0. They prepared cigarettes they normally smoked and attached them to the respective capsules as required. The smoking of capsule cigarettes was discretionary. The number of cigarette boxes opened each day, the number of cigarettes remaining in these boxes, and the number of cigarettes smoked other than the designated cigarettes were recorded in a diary distributed to the participants.

2.7 Measurement method

Blood pressure, pulse pressure, heart rate, baPWV, and ankle-brachial index were measured using FORM-5 (Fukuda Colin Co., Ltd., Tokyo, Japan) or BP-203RPEIII (Omron Corporation, Kyoto, Japan). The variables % vital capacity and %FEV1.0 were measured using the SP-3700 COPD Lung Per (Fukuda Denshi Co., Ltd., Tokyo, Japan) or HI-205 T (Chest M.I., INC., Tokyo, Japan) spirometer. Height and weight were measured using the AD-6351 or AD-6228AP (both A&D Company, Limited, Tokyo, Japan) scales. Carotid ultrasonography was performed using Viamo SSA-640 A (Toshiba Corporation, Tokyo, Japan) or HD3 (Philips Japan, Ltd., Tokyo, Japan). Chest radiographs were captured using the NAOMI-2002 (RF Co., Ltd., Nagano, Japan) or KXO-32 S (Canon Medical Systems Corporation, Otawara, Japan) systems. Electrocardiograms were measured using Vasera VS-1500ATW or ECG-1350 (both Fukuda Denshi Co., Ltd.). Pregnancy tests were

performed using an HCG Quick Checker Dip (Mizuho Medy Co., Ltd., Tosu, Japan). Blood and urine tests were outsourced to the LSI Medience Corporation (Tokyo, Japan), while the salivary cotinine test was outsourced to Hoken Kagaku, Inc. (Yokohama, Japan).

2.8 Sample size

Statistical significance and power were set at 5% bilaterally and 80%, respectively. Based on the results of a pilot study (20), the baPWV reduction in the BCP group was 73 cm/s, whereas the increase in the placebo group was 3 cm/s, with a standard deviation of 117 cm/s. Assuming a 10% dropout rate, the target population was calculated as 90 participants. Interim analyses were not performed.

2.9 Statistical analysis

Comparisons between intervention groups for baPWV change up to week 12 were examined using a two-way repeated-measures analysis of covariance with group, time point, their interaction term, and baPWV on day 0 as independent variables. The SAS statistical analysis software suite (version 9.4_M7, SAS Institute Japan Ltd., Tokyo, Japan) was used to compare the above. Means and 95% confidence intervals were calculated. Within-group comparisons were performed on day 0 and week 12 for each group. Two-sided significance was set at 5%. Statistical analyses of the secondary endpoints and stratified analyses other than the questionnaire were performed similarly to those for the primary endpoint. The questionnaire, SF-36, and number of cigarettes smoked per week were tabulated at each time point. The Mann-Whitney U test was used for between-group comparisons, and Wilcoxon's signed-rank test was used for before-after comparisons within each group. Spearman's rank correlation test was used for correlation analysis. Multiple comparisons were corrected using Bonferroni correction. Statistical analyses were performed by Satt Co., Ltd.

3. RESULTS

3.1 Capsule analysis

The capsules contained 19.3 mg of liquid, with 2.54 mg of BCP per capsule in the BCP group, and no BCP was detected in the placebo group.

3.2 Study population

Of 292 applicants whose baPWV was larger than 1,300 cm/s, 208 were excluded because they did not meet all eligibility criteria, such as respiratory dysfunction, carotid artery intimal thickening, and hypertension. The number of people excluded by reason for screening is described in Table S10. Subjects were excluded from the study based on screening tests; therefore, on the day 0 test, which was conducted eight weeks after the screening test, a few people met the exclusion criteria. The remaining 84 participants were randomized to BCP (assigned: n = 42, intervention: n = 36, analyzed: n = 33) or placebo (assigned: n = 42, intervention: n = 39, analyzed: n = 36) groups. Only individuals not subjected to capsule cigarette smoking intervention were excluded from the analysis; all data from participants whose medical data could be measured were included in the analysis. After obtaining fixed data and during statistical analysis, no participant was further excluded from the analysis in either the BCP or the placebo group. Missing values were not imputed. The flowchart of the study procedure is shown in Figure 1.

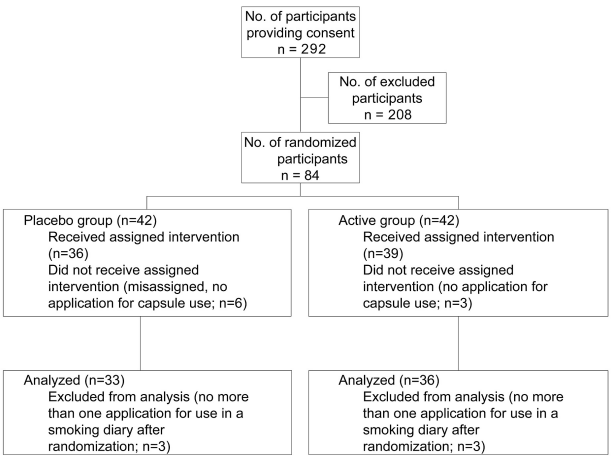

Figure 1. Flowchart of participant recruitment.

3.3 Baseline data

The baseline demographic and clinical characteristics of each group are presented in Table S11. A total of 208 individuals were excluded based on the exclusion criteria, leaving 84 participants (74 males and 10 females) with an average age of 48.9 ± 7.7 years old. Of these, 69 people were randomly assigned to either the BCP group (n = 36, 49.4 ± 8.7 years old) or the placebo group (n = 33, 49.9 ± 8.7 years old) for the commencement of a prospective trial. The major background factors did not differ significantly between the treatment groups. The cigarette brands smoked by individual participants with the nicotine and 'tar' amounts of these brands are listed in Table S3.

3.4 Outcomes

Figure 2 illustrates the blood concentrations of BCP and nicotine in each study group. In both groups, BCP concentrations were approximately 1 ng/mL before smoking. However, the BCP blood concentration reached a maximum concentration (Cmax) of 4.42 ng/mL at time-to-maximum (Tmax) of 10 min after starting to smoke in the BCP group. In contrast, no discernible increase in BCP blood concentration was detected in the placebo group (Table S12). The nicotine concentrations before smoking were 8.4 ng/mL in the BCP group and 5.2 ng/mL in the placebo group. The nicotine blood concentration curves at a Tmax of 10 min after starting smoking showed Cmax values of 15.64 ng/mL and 10.22 ng/mL in the BCP and placebo groups, respectively.

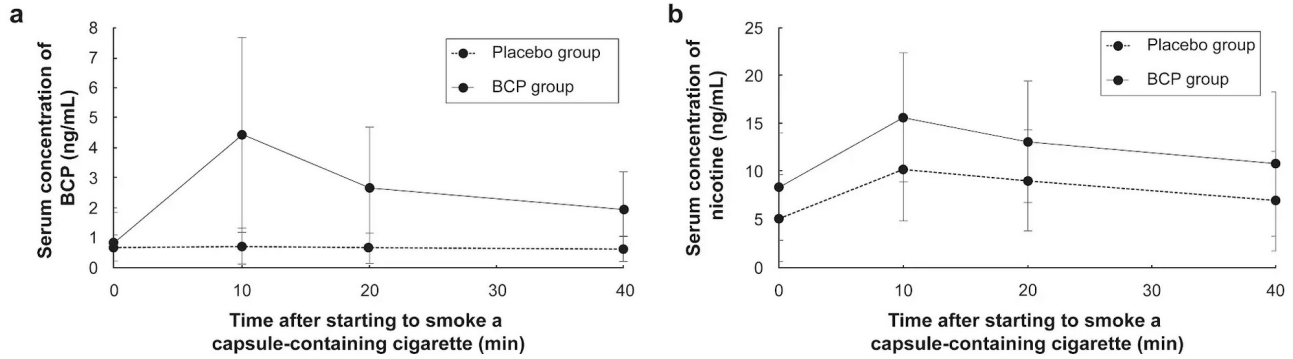

Figure 2. Changes in BCP (a) and nicotine (b) blood concentrations in the placebo and BCP groups before and 10, 20, and 40 min after smoking a capsule-containing cigarette. Error bars represent the standard deviation. BCP = β-caryophyllene.

A significant increase in nicotine blood concentration was observed in both groups (Table S13). The average nicotine amount per cigarette was 0.55 mg in the placebo group and 0.59 mg in the BCP group. Furthermore, the number of cigarettes smoked per day was 32.42–33.31 in the placebo group and 33.54–35.06 in the BCP group (Supplementary data 1). No significant difference in the number of cigarettes smoked per day or the rate of change in the number of cigarettes smoked was observed between the two study groups at any time point.

The mean absolute baPWV values on day 0 and at weeks 4, 8, and 12 (Table S14), the mean changes in baPWV (Table S15), and the before-after and between-group comparisons for baPWV changes through week 12 (Figure 3a, Table S16) were analyzed. The least-squares means of baPWV changes were 9.0 cm/s and −3.8 cm/s in the placebo and BCP groups, respectively. In the analysis of all subjects, the within-group and between-group comparison of baPWV change from day 0 to 4, 8, and 12 weeks showed no significant change in either group. The results related to secondary endpoints are described in the additional results (Supplementary data 2).

3.5 Stratified analysis

Although not included in the prior analysis plan, we performed exploratory stratified analyses of baPWV changes.

First, a stratified analysis was performed with a baPWV cutoff of 1,400 cm/s on day 0. Based on this, the absolute baPWV values on day 0 and at weeks 4, 8, and 12 (Table S17) and the before-after and between-group comparisons (Figures 3b, 3c, Table S18) were analyzed. In the group with a baPWV ≥ 1,400 cm/s on day 0, the within-group change in baPWV on day 0 did not differ significantly from that in the placebo group. However, significant differences were observed at weeks 4 (P = 0.003) and 8 (P = 0.038) in the BCP group (Figure 3c). The change in baPWV was also significantly different between the placebo and BCP groups at week 4 (P = 0.041; Figure 3c). Stratified analysis based on blood BCP levels was also performed, but no significant differences were found.

Moreover, correlation analyses were performed between the initial baPWV value and the baPWV change at 4, 8, and 12 weeks in the BCP and placebo groups (Figure 4, Table S19, Figure S2). No significant correlation was observed in the placebo group. However, the correlation coefficients in the BCP group were −0.502 (P = 0.002) at week 4, −0.615 (P < 0.001) at week 8, and −0.513 (P = 0.002) at week 12, indicating significant negative correlations.

3.6 Adverse events

Additional tables show the rates of adverse events in the placebo (n = 36) and BCP (n = 39) groups (Table S20, Table S21).

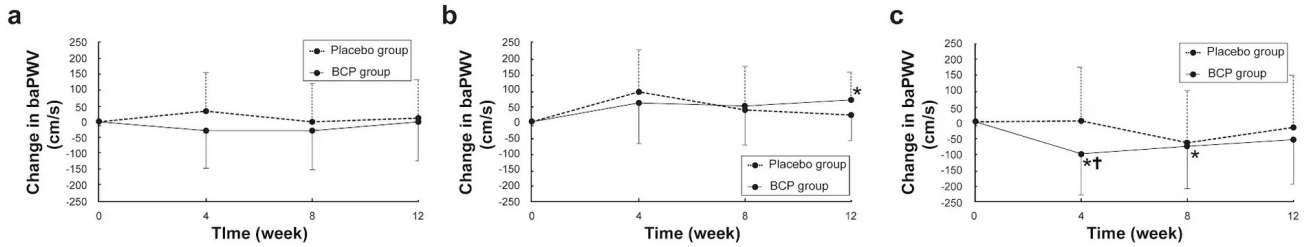

Figure 3. Changes in baPWV from day 0. (a) All participants. (b) Stratified analysis of participants with baPWV < 1,400 cm/s on day 0. (c) Stratified analysis of participants with baPWV ≥ 1,400 cm/s on day 0. Error bars represent standard deviations. *P < 0.05, within-group comparison *vs.* day 0; †P < 0.05: between-group comparison; baPWV: brachial-ankle pulse wave velocity; BCP: β-caryophyllene.

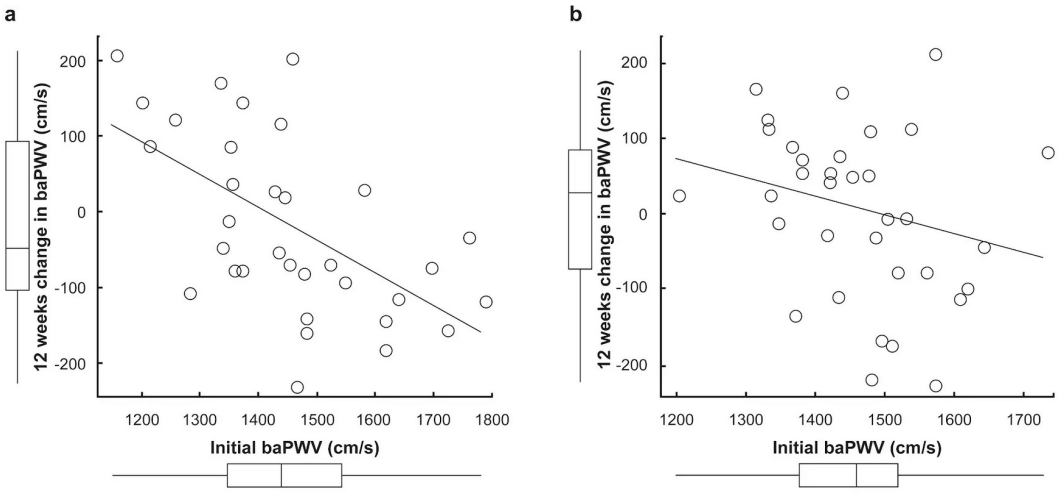

Figure 4. Correlations between baPWV on day 0 and the 12-week change in baPWV in the BCP (a) and placebo (b) groups.
baPWV: brachial-ankle pulse wave velocity; BCP: β-caryophyllene.

No serious adverse events were observed in either group. The incidence of adverse reactions was 2.8% (1/36 participants) for "increased sputum" and "oropharyngeal discomfort" in the placebo group and 2.6% (1/39 participants) for "increased sputum" in the BCP group, with no significant difference in the incidence of adverse reactions between the placebo and BCP groups. All adverse reactions resolved without treatment.

4. DISCUSSION

We hypothesized that adding volatile functional food ingredients with anti-inflammatory properties to flavor capsules in cigarette filters could reduce the risk of arteriosclerosis associated with smoking. To evaluate this, we incorporated BCP as a functional ingredient into capsules and assessed its impact on smoking. In the present study, we confirmed that smoking cigarettes with BCP-containing capsules resulted in the transfer of both BCP and nicotine into the bloodstream. The blood nicotine concentration was slightly higher in the BCP group than in the placebo group, but the difference was not statistically significant. Additionally, there was no difference in the number of cigarettes smoked per day between the two groups.

The BCP blood concentration range before smoking cigarettes containing test capsules was 0.2–1.8 ng/mL in the placebo group and 0.2–3.4 ng/mL in the BCP group. Notably, some participants initially had high blood BCP levels, possibly due to daily BCP intake via food. However, the BCP blood concentration in the BCP group at 10 min after starting to smoke was 0.2–13.3 ng/mL. This implies that some participants did not have elevated blood BCP concentrations despite using BCP-containing capsules. Although the reason remains unknown, some participants might have insufficient transpulmonary BCP ingestion, or individuals might differ in inhalation depth while smoking. Such participants would have less of an increase in blood BCP concentrations and would be less likely to benefit from BCP. However, because these factors were not the exclusion criteria for this study, all participants were included in the analysis.

In a past study, smoking cigarettes with BCP-flavored capsules resulted in a BCP Cmax of 4.2 ng/mL (20). Similarly, the BCP blood concentration in the present study was 4.42 ng/mL in the BCP group. Previous reports have also shown a Cmax of 12–15 ng/mL for 1–2 mg ingested nicotine (23). In the present study, the Cmax of nicotine was 10–16 ng/mL. In this study, we conducted a trial with healthy individuals who, under strict exclusion criteria, had borderline or slightly elevated baPWV (> 1,300 cm/s) values but otherwise exhibited normal respiratory function, cardiac function, blood pressure, and electrocardiogram results.

Arterial wall stiffness can be easily assessed using pulse wave velocity, a measure that estimates arterial stiffness by analyzing the speed at which the heart pulse travels through the artery (24). Although baPWV is affected by pulse rate (25) and systolic blood pressure (26), a baPWV of 1,400 cm/s corresponds to an intermediate risk according to the Framingham Risk Score (27) and an increased risk of developing hypertension (28), which has clinical significance as a cardiovascular risk level for which lifestyle improvements are recommended. Additionally, studies have reported that baPWV is affected by food (29) and drugs, including statins (30, 31). In the analysis of all participants in this study, the BCP group showed a decreasing trend for baPWV change compared with the placebo group; however, this difference between groups was not statistically significant, possibly due to the combined analysis of participants with normal and high baPWV. Although some secondary endpoints tended to be more favorable in the BCP group, they did not reach statistical significance.

In the stratified analysis, baPWV changes differed significantly among the participants with high baPWV values. The baPWV cutoff of 1,400 cm/s was based on the fact that a baPWV value of 1,400 cm/s or higher suggests the progression of arteriosclerosis and vascular stiffening. TANAKA *et al.* (32) used this value in other studies as a

cutoff to distinguish mild from moderate sclerosis (33–35). Accordingly, our results suggest that BCP inhalation lowers baPWV in healthy participants with potentially mild-to-moderate arterial stiffness (baPWV > 1,400 cm/s). This might explain why the analysis of all participants showed no significant differences, as BCP may not further lower the baPWV of participants with initially normal baPWV values. This is also evident from the results of the stratified analysis, showing an inverse correlation between the baPWV value on day 0 and baPWV changes after 12 weeks in the BCP group. From these results, we infer that regular inhalation of BCP could have protected regular smokers from the development of high baPWB.

In previous clinical studies, the difference in baPWV change between placebo and eicosapentaenoic acid (EPA) groups was 50 cm/s after 1 year of EPA intake (1,800 mg/day) (36). Another study on fluvastatin reported a 150 cm/s reduction in baPWV from 1,800 cm/s to 1,650 cm/s over one year (30). In the present study, the baPWV-lowering effect of BCP inhalation started at week 4 and was maintained until week 12. This decrease was in the range of 30–170 cm/s. This BCP effect was more potent than that of oral EPA intake and comparable to that of statins, even with continuous nicotine intake by smoking.

The effects of BCP inhalation on respiratory function were investigated by measuring the % vital capacity and %FEV1.0 (Supplementary data 2). However, no significant changes were detected in within-group or between-group comparisons, indicating that respiratory function was unaffected. Moreover, blood or urine test results did not significantly change in either group and the incidence of increased sputum production, an adverse event observed in the BCP group, did not differ between the BCP and placebo groups. Thus, smoking cigarettes with BCP capsules may have anti-arteriosclerotic effects without raising safety concerns.

Regarding the possible mechanism of BCP action, animal studies have shown that BCP improves vascular mechanical strength and flexibility by reducing elastic fiber breakdown due to less inflammation in blood vessels (19). However, in the present study, the levels of the inflammatory markers high-sensitivity C-reactive protein and fibrinogen did not decrease in the BCP group, indicating that the anti-inflammatory effects of BCP may be organ-specific rather than systemic. There is no report that BCP counteracts the inflammatory effects due to specific components in cigarette smoke (nicotine, 'tar', etc.). Further studies are needed to verify the anti-inflamatory effect of BCP to cigarette smoke. Another possible explanation for the individual differences in BCP efficacy might be the interaction between the BCP and CB2 receptors. In animal studies, CB2 receptor antagonists competitively inhibited the effects of BCP on blood vessels. Similarly, in humans, CB2 receptors likely mediate BCP effects as well; however, the expression levels of CB2 receptors on immune cells differ among individuals (37). Moreover, as BCP is also a ligand for PPARs, unreported additional pathways may exist. Furthermore, BCP administration in rats maintained the vascular eNOS–iNOS balance and restored NO levels (38), indicating enhanced aortic flexibility via vascular endothelial function. Although blood pressure affects baPWV (39), the blood pressure did not change in the present study, whereas baPWV decreased, suggesting that this decrease was not a secondary effect associated with lower blood pressure but rather with a reduction in vascular stiffness and improved flexibility.

Nonetheless, this study had some limitations.

- First, the participants were asked to attach the capsule to a cigarette filter by themselves and to self-report the number of cigarettes they smoked. Given the tedious nature of attaching the capsule each time the participant wanted to smoke, participants in the BCP group might not have reported smoking without an inserted capsule. Therefore, the observed BCP effect may have been underestimated. This is supported by the fact that previous studies (20), in which capsule cigarettes were provided showed significant differences before and after comparisons at weeks 4, 8, and 12.
- Second, regarding adjustment for multiple comparisons, a significance level of 5% may not be appropriate for blood and urine test results. However, multiplicity was considered in the baPWV analyses.
- Third, this study was limited to healthy participants. Thus, the potential effects of BCP on individuals with prevalent diseases remain unknown.
- Fourth, the observation period was only 12 weeks, and long-term studies are required to determine the long-term effects of BCP capsules in cigarettes.
- Fifth, all participants in this study were Japanese. Therefore, when extrapolating the study results to non-Japanese individuals, it is necessary to consider that their usual BCP intake may differ from that of Japanese individuals.

BCPs were also detected in the blood of the participants in the placebo group, possibly representing dietary or environmental BCPs. Thus, the BCP effects observed in this study may also be influenced by other lifestyle habits, such as daily exercise and sleep.

Furthermore, BCP was taken up via the transpulmonary route in the present study; however, it might also be ingested with food; thus, the described effects might also be observable in non-smokers. At this stage, it is difficult to predict how much of the toxic effects of smoking can be offset by smoking cigarettes with a BCP capsule, and consequently, how much the harm to health can be reduced, based on the relative relationship between the toxicity of tobacco and the therapeutic efficacy of BCP. Further research on this matter is necessary.

5. CONCLUSIONS

Smoking cigarettes with BCP-containing capsules resulted in transpulmonary BCP uptake into the blood, thereby lowering baPWV in individuals with high baPWV. This idea can be used not only for capsules for cigarettes, but also for heat-not-burn tobacco or e-cigarettes. Our data suggest that this approach is expected to benefit public health.

GLOSSARY

- β-Caryophyllene (BCP):
  A naturally occurring sesquiterpene found in many essential oils, known for its anti-inflammatory and analgesic properties.

- Aortic stiffness:
  A measure of the rigidity of the aorta, the main artery carrying blood from the heart to the rest of the body; increased aortic stiffness is a risk factor for cardiovascular diseases.
- Brachial-ankle pulse wave velocity (baPWV):
  A measure of arterial stiffness calculated by assessing the speed at which blood pressure pulses travel between the brachial artery in the arm and the ankle arteries.
- Two-Way Repeated-Measures Analysis of Covariance (RM-ANCOVA):
  A statistical method used to analyze the differences between groups over multiple time points while controlling for one or more covariate(s).
- Sesquiterpene:
  A class of terpenes consisting of three isoprene units, often found in plants and contributing to their aroma and potential therapeutic properties.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethics Review Committee of Hillside Clinic Jingumae on November 2, 2022 (approval number: HR08923). Consent for study participation was obtained from all participants in writing after explaining the study details in a manner that was sufficiently clear and easy to understand, using an explanatory document approved by the Ethics Review Committee.

CONSENT FOR PUBLICATION

Not applicable

AVAILABILITY OF DATA AND MATERIALS

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

COMPETING INTERESTS

KY and YM are employees of Sunsho Pharmaceutical Co., Ltd. NT, YY, and SM are employees of Inabata Koryo Co., Ltd. Kindai University, Inabata Koryo Co., Ltd., and Sunsho Pharmaceutical Co., Ltd. applied for patents related to this research (WO/2021/182538, WO/2022/097601, WO/2023/042819, WO/2023/042820, and WO/2023/210481). BCP was provided at no charge by Inabata Koryo Co., Ltd. The remaining authors declare that they have no competing interests.

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AUTHORS' CONTRIBUTIONS

KY conceptualized and designed the study and was a major contributor in writing the manuscript. YM supervised the research and reviewed and edited the manuscript. NT, YY, and SM analyzed capsule core and serum samples using gas chromatography–mass spectrometry, provided raw materials, and reviewed and edited the manuscript. NZ, MH, and NU conceptualized and designed the study, and reviewed and edited the manuscript. All authors read and approved the final manuscript.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

We did not use any AI and AI-assisted technologies in the writing process.

TRIAL REGISTRATION

This clinical trial ("Investigation of the effectiveness of inhalation intake of beta-caryophyllene against vascular wall stiffening caused by smoking") was prospectively registered on December 3, 2022, under the UMIN number UMIN000049675.

SUPPLEMENTARY INFORMATION

This publication contains supplementary material available at: https://sciendo.com/de/article/10.2478/cttr-2025-0011.

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