Pharmacological Research 97 (2015) 1–6

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Platelet aggregation values in patients with cardiovascular risk factors are reduced by verbascoside treatment. A randomized study

Gianluca Campo[a,b,*], Rita Pavasini[a], Simone Biscaglia[a], Alessandra Ferri[a], Elisa Andrenacci[a], Matteo Tebaldi[a], Roberto Ferrari[a,b,c]

[a] Cardiovascular Institute, Azienda Ospedaliero-Universitaria S. Anna, Cona, FE, Italy
[b] Laboratorio per le Tecnologie delle Terapie Avanzate (LTTA) Center, Ferrara, Italy
[c] Maria Cecilia Hospital, GVM Care & Research, E.S. Health Science Foundation, Cotignola, Italy

ARTICLE INFO

Article history:
Received 5 February 2015
Received in revised form 26 March 2015
Accepted 26 March 2015
Available online 3 April 2015

Keywords:
Aspirin
Platelet aggregation
Light transmission aggregometry
Verbascoside
Cardiovascular risk factors

ABSTRACT

Verbascoside, a phenolic compound, showed several favorable biological activities, including an antiplatelet activity. No in vivo studies tested its efficacy and safety in subjects with cardiovascular (CV) factors. The aim of this randomized, single-center, double-blind, phase II study was to assess the efficacy and tolerability of verbascoside intake for the modulation of platelet aggregation (PA) values in subjects with cardiovascular (CV) risk factors. One-hundred subjects with at least one CV risk factor (age >65 years, diabetes mellitus, hypertension, current cigarettes use, hyperlidemia, waist circumference >102 cm in male or >88 cm in female) were enrolled and randomly assigned to receive placebo or verbascoside 50 mg or verbascoside 100 mg. PA was measured at baseline and after 2 weeks of study drug assumption, with light transmittance aggregometry (arachidonic acid, AA, 1 μM and adenosine diphosphate, ADP, 5 μM). Two weeks of treatment with placebo or verbascoside 50 mg did not modify PA values (both after AA and ADP stimuli). On the contrary, after 2 weeks of verbascoside 100 mg, PA values decreased significantly (from 51 ± 13% to 39 ± 15%, $p < 0.01$ after AA; from 60 ± 12% to 49 ± 15%, $p = 0.01$ after ADP). No serious adverse events were reported during the study, and no subjects discontinued the study because of adverse events.

We conclude that long-term intake of verbascoside 100 mg significantly reduces PA values in subjects with CV risk factors.

1. Introduction

Complementary and alternative medicine (CAM) is a group of different medical and healthcare systems, practices, and products that are not generally considered part of conventional medicine [1]. Biologically based therapies (e.g., herbal treatments, mega-dose vitamins) are one of the 5 categories or domains of CAM, as classified by the National Center of Complementary and Alternative Medicine [2]. CAM has become increasingly popular in the United States and Europe during the past few years. A recent survey showed that 82.5% of the outpatients with cardiovascular disease (CVD) reported use of CAM therapies [1]. Biologically based therapies and dietary supplements are among the most commonly used CAM modalities in patients with CVD [1–3]. These products have become largely accepted as a part of the treatment for elevated serum cholesterol and/or triglycerides and for the maintenance of vascular wall health [4]. Verbascoside is a polyphenol compound with antioxidant, wound healing and cardio-protective actions [5]. Using blood samples of healthy volunteers with cardiovascular risk factors, we previously demonstrated that verbascoside is able to modulate in vitro platelet aggregation (PA) triggered both by arachidonic acid (AA) and adenosine diphosphate (ADP) [6].

The aim of this randomized trial was to assess efficacy and tolerability of verbascoside in the modulation of PA values in primary cardiovascular (CV) prevention. Therefore, subjects with CV risk factors were enrolled and randomly assigned to receive verbascoside or placebo.

Abbreviations: AA, arachidonic acid; ADP, adenosine diphosphate; PA, platelet aggregation; CV, cardiovascular; CVD, cardiovascular disease; CAM, complementary and alternative medicine; PRP, platelet rich plasma; PPP, platelet poor plasma; IPA, inhibition of platelet aggregation; PD, platelet disaggregation; CoV, cofficient of variability.

* Corresponding author at: Cardiovascular Institute, Azienda Ospedaliero-Universitaria S. Anna, Via A. Moro 8, Cona, FE, Italy. Tel.: +39 0532237079; fax: +39 0532241885.
E-mail address: cmpglc@unife.it (G. Campo).

http://dx.doi.org/10.1016/j.phrs.2015.03.020
1043-6618/

2. Methods

2.1. Study design and population

This is a randomized, single-center, double-blind, phase II study designed to evaluate the superiority of verbascoside (50 mg or 100 mg) as compared to placebo in the modulation of PA values of subjects with at least one CV risk factor. We recruited subjects from cardiology outpatients department in Ferrara, Italy. The institutional review board of Ferrara approved the trial, and all subjects gave written informed consent. Subjects were eligible if they were >18 years and had at least one of the following CV risk factors: age >65 years, diabetes mellitus, hypertension, current cigarettes use, hyperlipidemia, waist circumference >102 cm in male or >88 cm in female. We excluded subjects if they were in medical treatment with antiplatelet agents (aspirin, clopidogrel, ticlopidine, prasugrel, ticagrelor), or with anticoagulant drugs (e.g. warfarin), or with oral contraceptive. Other exclusion criteria were: history of myocardial infarction, coronary revascularization (percutaneous or surgical), stroke, transient ischemic attack, peripheral vascular disease and recent (last month) assumption of corticosteroids and/or non-steroidal anti-inflammatory drugs.

2.2. Randomization and interventions

Fig. 1 shows the study flow-chart. We recruited subjects between June 2013 and December 2013. 144 subjects were screened; 44 were ineligible for the study or declined to participate. One-hundred subjects were enrolled and randomly assigned (Table 1) to receive verbascoside 25 mg twice daily (verbascoside 50 mg group), verbascoside 50 mg (two 25 mg tablets) twice daily (verbascoside 100 mg group), or two placebo tablets twice daily (placebo group) for 2 weeks. Randomization was performed by an independent study coordinator via sealed envelopes. We randomly assigned subjects in blocks of 4, stratified according 4 categories: age >60 vs. ≤60 years, male vs. female sex, current use of cigarettes (yes vs. no) and diabetes mellitus (yes vs. no). In fact, age, sex, smoke, and diabetes significantly affect PA values and had to be balanced between the three treatment groups [7–10]. At the end of the study, we assessed treatment adherence by the self-report of number of missed doses per week. Both patients and study-team members were blinded to treatment allocation throughout the 2 weeks of treatment.

2.3. Blood samples

Venous blood samples were collected at baseline (before the study drug administration) and at the end of week 2, to assess potential differences between groups before and after treatment and to assess response to verbascoside within each population (Fig. 1). Blood samples for platelet function assays were collected from an antecubital vein using a 21-gauge needle. The first 2–4 mL of blood were discarded to avoid spontaneous platelet activation. All subjects underwent blood sampling after at least 30 min of rest and 2 h of fasting. Two blood samples (4.5 mL tubes containing 3.8% sodium citrate) were collected from each participant.

2.4. Platelet function analysis

PA was performed with light transmittance aggregometry in all patients according to standard protocols [11,12]. Blood was centrifuged ($200 \times g \times 10$ min) to obtain platelet-rich plasma (PRP). The remaining specimen was re-centrifuged ($1500 \times g \times 15$ min) to obtain platelet-poor plasma (PPP). Platelets were stimulated with AA 1 μM and ADP 5 μM. Aggregation was measured at 37 °C with a PACKS-4 Aggregometer (Helena Laboratories). Curves were recorded for 6 min. Aggregation was measured at maximal aggregation (maximal PA) and at 5 min (late PA). Inhibition of platelet aggregation (IPA) was defined as the percent decrease in aggregation values (maximal PA and late PA) obtained at baseline and after treatment: IPA (%) = (PA at baseline − PA at week 2)/PA at baseline. Percentage of platelet disaggregation (PD) between maximal PA and late PA values was defined as: PD (%) = 100 × (1 − late PA/maximal PA). To define whether significant interindividual variability was present in response to antiplatelet treatment, the coefficient of variability was used (CoV = standard deviation (SD)/mean). Significant variability was defined when the CoV was >0.25 in continuous variables with a normal distribution, as previously described [13].

2.5. Capsules packaging and verbascoside extraction

We received both verbascoside and placebo capsules directly from the manufacturer (Istituto di Ricerche Biotecnologiche S.p.A., Vicenza, Italy). They were identical in size, shape, and color. Participants received a 21-day supply of study product. The procedure to obtain verbascoside has been previously described [6]. Briefly, verbascoside was obtained by cell line of a *Syringa vulgaris* plant from the Botanical Garden of the University of Bologna (Bologna, Italy). This highly selected cell line for the synthesis of verbascoside is deposited at the Plant Cell Bank (DSMZ, Deutsche Sammlung Von Mikroorganismem un Zellkulturen, Braunschweig, Germany) coded internally IRB SV25/B and internationally DSM 16857.

2.6. Outcome, follow-up, tolerability and safety

The primary outcome was maximal PA values after 2 weeks of treatment with the study drug. Secondary outcomes were the following PA evaluations after 2 weeks of treatment with the study drug: late PA, %IPA, %PD. All subjects who were enrolled in the study and received at least one dose of study drug were included in the safety analyses. Any potential side effect was recorded regardless of the relation to study drug or study procedures. Routine laboratory measurements (e.g. hematology and clinical chemistry) and vital signs were measured at baseline and at follow-up (week 2). Discontinuations due to adverse events were also recorded.

2.7. Statistical analysis

According to previous studies, we hypothesized baseline PA values of 50–60% with a SD of 10–12% [6–13]. Assuming that the use of verbascoside would decrease PA by 10% vs. placebo, at least 30 patients per group were required for 85% power and a 2-sided α-value of 0.05. Continuous data were presented as mean ± SD and were tested for normal distribution with the Kolmogorov–Smirnov test. The variables normally distributed were compared by *t* test and one-way ANOVA; otherwise the Mann–Whitney *U* and Kruskal–Wallis tests were used. Of note, maximal PA values (primary outcome) were normally distributed. Categorical variables were summarized in terms of numbers and percentages and were compared by using the two-sided Fisher's exact test. *p*-Value was considered significant if <0.05. All analyses were performed with STATISTICA 8 (Statsoft Inc, Tulsa, Okla, USA).

3. Results

Table 1 shows the baseline characteristics of enrolled subjects. Participants were Caucasian middle-aged men and women. The mean age was 62.5 ± 6 years. Forty (40%) of the 100 participants were female. We enrolled 31 (31%) subjects affected by diabetes mellitus. More than 90% of subjects showed at least 2 CV risk factors (Table 1). According to the high frequency of risk factors, we