Fitoterapia 82 (2011) 933–938

Contents lists available at SciVerse ScienceDirect

Fitoterapia

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

Hypoglycemic effect of lupin seed γ-conglutin in experimental animals and healthy human subjects

Juan C. Bertoglio [a], Mario A. Calvo [a], Juan L. Hancke [b], Rafael A. Burgos [b], Antonella Riva [c], Paolo Morazzoni [c], Cesare Ponzone [c], Chiara Magni [d], Marcello Duranti [d,*]

[a] Instituto de Medicina, Valdivia, Chile
[b] Instituto de Farmacologia Universidad Austral de Chile,Valdivia, Chile
[c] Indena S.p.A., Milan, Italy
[d] Dept. of AgriFood Molecular Sciences, Università degli Studi di Milano, Italy

ARTICLE INFO

Article history:
Received 18 March 2011
Received in revised form 6 May 2011
Accepted 8 May 2011
Available online 14 May 2011

Keywords:
Antidiabetic
Lupinus albus
Protein extract
Animal trial
Human study

ABSTRACT

A lupin seed γ-conglutin-enriched preparation was tested in a glucose overload trial with both murine models and adult healthy volunteers. The results with rats showed a dose-dependent significant decrease of blood glucose concentration, which confirmed previous findings obtained with the purified protein. Moreover, three test-product doses equivalent to 630, 315, and 157.5 mg γ-conglutin, orally administered 30 min before the carbohydrate supply, showed a relevant hypoglycemic effect in human trials. Insulin concentrations were not significantly affected. The general hematic parameters did not change at all.
This is the first report on the glucose-lowering effect of lupin γ-conglutin in human subjects.
© 2011 Elsevier B.V. All rights reserved.

Introduction

Hyperglycemia is recognized to be the central feature of all unbalances in the metabolism of carbohydrates, lipids, ketones and amino acids [1]. The most diffused pathological condition, characterized by stable hyperglycemia, is known today as type-2 diabetes (accounting for about 90% of all diabetes cases). Nowadays, type-2 diabetes is considered an epidemic disease, especially in the Western countries, where the incidence in the population is estimated to range from 2% up to 4% [2]. Type-2 diabetes is usually preceded by years of an abnormal condition, termed impaired glucose tolerance (IGT) characterized by plasma glucose levels between 140 and 199 mg/dL, 2 hours after a standard oral glucose challenge, but not as high as in diabetes (>200 mg/dL). Several characteristics in the population have been recognized to be associated with a greater risk of progression from IGT to type 2 diabetes. Among these are impaired insulin secretion, insulin resistance, obesity and age [2–4]. Therefore, actions aimed at controlling this situation are crucial.

Lupin is a leguminous seed which has largely been used as a food for its high protein content in the Mediterranean area since thousand years. Around the year 1000 AD, the Persian doctor Ibn Sīnā, among the firsts to describe diabetes symptoms, used mixtures of lupin, fenugreek and zedoary seed flours to remarkably reduce sugar excretion. Lupin seed is mentioned in the ancient and traditional pharmacopoeia books as an anti-diabetic product. Last century, in the search of the lupin seed active principle, Orestano described the extraction and purification from white lupin seeds of a compound capable of decreasing glycemia in rabbits. However, the extraction was tedious and the yield extremely low and thus the product was considered to be lacking of any

Abbreviations: BMI, body mass index; BUN, blood urea nitrogen; GOT, glutamate-oxaloacetate transaminase; GPT, glutamate-pyruvate transaminase; GGT, γ-glutamyl transpeptidase; LDH, lactate dehydrogenase.

* Corresponding author at: Department of AgriFood Molecular Sciences, Università degli Studi di Milano, Via Celoria, 2 I-20133 Milano, Italy. Tel.: +39 02 503 16817; fax: +39 02 503 16801.
E-mail address: marcello.duranti@unimi.it (M. Duranti).

0367-326X/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.fitote.2011.05.007

potential application [5]. The possibility that a lupin seed protein could be the active principle was not taken in consideration until recently.

During our research activities on lupin seed proteins, we undertook a series of research activities aimed at unraveling the specific hypoglycemic role of one of the lupin proteins, termed γ-conglutin. Lupin γ-conglutin is a homo-tetrameric glycoprotein in which the monomeric unit consists of two disulphide linked heterogenous subunits of about 30 and 17 kDa (for a review on γ-conglutin properties, see reference 6). From the viewpoint of γ-conglutin biological activity, orally-administered pure γ-conglutin was found to effectively decrease plasma glucose in glucose overloaded rats in a dose-dependent manner [7]. More recently, the insulin-mimetic activity of γ-conglutin on differentiating myoblasts was described [8].

In this work, the remarkable glucose lowering capacity of a lupin γ-conglutin-enriched preparation was confirmed in rat models and assessed for the first time in healthy human subjects.

2. Materials and methods

2.1. Lupin seed γ-conglutin laboratory purification

Lupinus albus L. seed γ-conglutin was purified to homogeneity from dehulled dry lupin seeds by using a combination of chromatographic steps, as described by Duranti et al. [9].

2.2. Lupin seed γ-conglutin preparation for animal and human studies

A lupin γ-conglutin-enriched dry extract (Pro-Gamma™) was prepared by INDENA S.p.A., Milano, Italy, according to an industrially-developed procedure (WO 2004/071521) which consisted essentially in a protein wet extraction process and solvent precipitation. The test product for the study was manufactured in the form of dried powder. The protein content of the dry powder, as assessed by the Lowry method [10], was 44.8% of the dry weight. γ-Conglutin content, evaluated by SDS-PAGE densitometric scanning, was about 47% of the total proteins in the dry powder (see below).

The test product was used as such in rat studies at the dosages of 50, 100 and 200 mg/kg b.w. (see below details on the administration procedure). As far as the human trials are concerned, the dry powder was packed in vacuum sealed sachets, for easy handling, containing 1500 mg powder each (equivalent to 315 mg of γ-conglutin) by FARMINDUSTRIA S.A. Laboratories (Santiago, Chile). For placebo, the formula was the same as for the *verum* formulation, except for the exclusion of lupin dry extract, which was substituted by microcrystalline cellulose (Avicel PH302). The test product and the placebo were kept at room temperature in a dry place and protected from light.

2.3. SDS-PAGE

SDS-PAGE was carried out on 12% polyacrylamide gels, according to Laemmli [11] under reducing and non-reducing conditions using a mini-PROTEAN II cell (Bio-Rad). The gels were Coomassie blue stained.

The SDS-PAGE images were acquired using a scanner, Canoscan 8000F (Canon, Milan, Italy) interfaced with a personal computer and densitometric scans were carried out with ImageMaster 1D software (Amersham Pharmacia Biotech, Milan, Italy).

2.4. Design of the animal study

A total of 100 male rats (Charles River, Calco, LC, Italy) with an average body weight ranging between 275 and 300 g were maintained under stable conditions for 7 days before the experiment. The animals were given a standard rat diet and were kept under automatically controlled light, temperature, and humidity conditions. The rats were divided into five groups. One group, the control group, received only the control product, i.e., the placebo; three groups received 50, 100, or 200 mg/kg body weight of the γ-conglutin-enriched test product, corresponding to 10.5, 21.0 and 42.0 mg γ-conglutin; the last group was given 50 mg/kg body weight of metformin added to the control product. Administration was carried out by gavage, 30 min before the glucose overloading experiment. At time 0 of the experiment, each rat was given 2 g/kg body weight glucose administered orally. At established times thereafter (0, 30, 60 and 90 min from glucose administration), each rat in a group of 5 rats per time and dose was treated with 50 mg/kg body weight Na-thiopenthal, and 5 mL of blood were collected in 7.5 mmol/L EDTA containing tubes. The blood was immediately centrifuged at 2000×*g* at 4 °C for 10 min and the supernatant used for glucose assays. All procedures involving rats and their care were performed according to the Italian Government Guidelines for animal tests and were in agreement with the European Commission rules (86/609/EEC).

2.5. Design of the human trial

The present trial was a placebo-controlled study conducted on fifteen adult healthy volunteers, who received three different single test doses of respectively 750 (half the content of 1 sachet), 1500 (1 sachet) and 3000 mg (2 sachet) of the test product, corresponding to 157.5, 315 and 630 mg γ-conglutin, respectively, and a placebo, with no γ-conglutin added. The whole duration of the trial was 7 weeks. The three doses and the placebo were administered *per os* 30 min before a carbohydrate meal, consisting in one serving of 85 g boiled (Grade 1) white rice which corresponded to an intake of 75 g carbohydrate. The flowchart of the clinical study is reported in Table 1.

The study was organized and directed by Patagonia Clinical Trials, CRO program of the Institute of Pharmacology, Universidad Austral de Chile in the city of Valdivia, Chile. All the volunteers were recruited from the city of Valdivia. The study started after approval by the local Bioethical Committee with the recruitment of the volunteers and was completed within 5 weeks from the beginning of the first session. Written informed consent was obtained from all subjects.

2.6. Recruitment of the subjects

Fifteen healthy adult (>18 years old) male and female volunteers, who complied with the inclusion criteria, were

Download English Version:

https://daneshyari.com/en/article/5831533

Download Persian Version:

https://daneshyari.com/article/5831533

Daneshyari.com