SHORT COMMUNICATION

Chenopodin as an anti-inflammatory compound

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ABSTRACT
Chenopodin is an 11S-type globulin purified from Chenopodium quinoa seeds, which can bind carbohydrates and hemagglutinating human erythrocytes. The present study aimed to evaluate the N-terminal structure of the heterodimeric Chenopodin and its effects in models of inflammation. Chenopodin presented two subunits on its structure and has N-terminal homology with other Chenopodin in 92%. Chenopodin decreased paw edema and neutrophil recruitment induced by carrageenan in mice. Concluding, we demonstrated that Chenopodin exhibits in vivo anti-inflammatory activity.

ARTICLE HISTORY
Received 4 June 2021
Accepted 6 September 2021

KEYWORDS
Chenopodin; Chenopodium quinoa; seeds; anti-inflammatory activity; in vivo

1. Introduction

Chenopodium quinoa is an Andean crop that grows in different environments as it can germinate in various altitudes and climates, resisting drought, cold temperature, and poor soil. Despite in vitro digestibility studies with C. quinoa extract indicated the presence of anti-nutritional factors as inhibitors of protease and lectins (Silva et al. 2015),
seeds present some interesting nutritional properties due to its high protein content with many essential amino acids (Graf et al. 2015). The C. quinoa genome has been recently published, enabling studies of the biological use of proteins obtained from this species (Jarvis et al. 2017). The major seed storage protein of C. quinoa, an 11S-type globulin, was isolated by Brinegar and Goundan (1993) and named Chenopodin. Chenopodin can bind carbohydrates, hemaglutinates human erythrocytes and also exhibits activity against Gram-negative bacteria strains such as Escherichia coli, Pseudomonas aeruginosa and Salmonella enterica (Pompeu et al. 2015).

Another peptide isolated from C. quinoa seeds, lunasin, also exhibits reactive oxygen species scavenging activity and inhibits the production of nitric oxide, tumor necrosis factor (TNF)-α and interleukin-6 by RAW264.7 macrophages in vitro (Ren et al. 2017). It has also been reported that extracts obtained from other species of Chenopodium, including C. album and C. ambrosioides, exhibit activity in in vivo models of inflammation and pain (Ajayi et al. 2017). Preliminary results indicating that peptides released from C. quinoa protein may act as scavengers of reactive oxygen species (Vilcacundo et al. 2018), molecules that contribute to the development of inflammation (Nagata 2005), prompted us to investigate the N-terminal sequence of the two subunits of Chenopodin and its effect in acute experimental models of inflammation, including the effects on paw edema, neutrophil migration and production of TNF-α.

2. Results and discussion

Chenopodin is a heterodimeric protein with two subunits. Analysis of the N-terminal alignment of the purified protein (Chenopodin subunit 1) with other proteins published in the Protein Data Bank revealed 92% homology with the previously published Chenopodin, which was extracted and purified from another variety of C. quinoa (Figure S1) and only 67% with Globulin 11S from Amaranthus. It was not possible to carry out the homology comparison of Chenopodin subunit 2 due to the termination of N-terminal sequencing when a proline residue at position 5 was detected.

Previously studies demonstrated that Chenopodin is a heterodimeric protein of 60 kDa, consisting of two subunits of approximately 25 kDa and 35 kDa with hemagglutinating activity in human erythrocytes. It is remained stable under a wide range of pH levels and temperatures (Pompeu et al. 2015). Chenopodin used in the present study differs from the 11 s-type globulin extracted and purified by Brinegar and Goundan (1993) by 11%. This difference may be due to the use of different varieties of C. quinoa. The seeds used in our study were cultivated in the Brazilian Savannah, unlike the seeds used in study carried out by Brinegar and Goundan (1993), which were obtained in an American market, without indication of origin. Different varieties of the same species may have small differences in genotypes (Spehar and Santos 2005), which could explain the fact that the protein evaluated in our study exhibited a slight difference in its N-terminal structure. In addition, portion 1 of Chenopodin demonstrated 74% homology with prunin 2 (PDB ID 3FZ3.A) and only 67% homology with Globulin 11S from Amaranthus.

To investigate whether Chenopodin exhibits anti-inflammatory activity in experimental models of acute inflammation, we evaluated effects in the models of paw
edema and pleurisy induced by carrageenan in mice. Treatment with Chenopodin (0.1, 1, and 10 mg/kg, i.v., −30 min) inhibited the paw edema induced by carrageenan at 2 and 4 h following injection of the inflammatory stimulus (Figure S2). Next, we investigated whether Chenopodin inhibits leukocyte recruitment in a pleurisy model. As shown in Figure S3, previous administration (30 min) of the highest dose of Chenopodin (10.0 mg/kg, i.v.) reduced both the neutrophil (Figure S3A) and total leukocyte (Figure S3B) recruitment induced by carrageenan. However, lower doses of Chenopodin (0.1 or 1.0 mg/kg, i.v.) failed to induce this effect. We also investigated whether the anti-edematogenic activity of Chenopodin was associated with reduced production of TNF-α. The intraplantar injection of carrageenan induced an increase of TNF-α concentration measured in the paw tissue after 4 h. Previous administration of Chenopodin (10.0 mg/kg, i.v.) did not alter the production of TNF-α induced by carrageenan (Figure S3C).

As peptides released from C. quinoa protein may act as scavengers of reactive oxygen species, molecules that contribute to the development of inflammation (Nagata 2005), we investigated the effects of Chenopodin in acute experimental models of inflammation. Initially, we investigated the effects induced by Chenopodin in the paw edema model induced by carrageenan. Intraplantar injection of carrageenan induced a marked inflammatory response, characterised by edema. This acute response is mediated by neutrophil migration and activation and production of many inflammatory mediators that induce an increase in vascular permeability and vasodilatation (Rocha et al. 2006). A single dose of Chenopodin inhibited the inflammatory edema induced by carrageenan. This effect may be due to reduced production of inflammatory mediators. As TNF-α is an important cytokine that mediates many aspects of the inflammatory response (Rocha et al. 2006), we evaluated the effects induced by Chenopodin on its production following intraplantar injection of carrageenan. Chenopodin failed to reduce TNF-α concentration in the paw tissue of animals, indicating that an effect on the production of other inflammatory mediators more likely explains its anti-edematogenic activity.

We next investigated the effects induced by Chenopodin in an experimental model of pleurisy in mice. The pleurisy model induced by carrageenan was used to evaluate leukocyte recruitment, mainly neutrophils, and the production of mediators that play an important role in acute inflammation (Luchese et al. 2012). In the present study, neutrophil recruitment induced by carrageenan was inhibited by treatment with Chenopodin. Thus, the present data suggest that the in vivo anti-inflammatory activity of Chenopodin may be due inhibition of the production or action of inflammatory mediators other than TNF-α, which in turn may underlie its marked effect on neutrophil recruitment. Although other components from C. quinoa seeds such as polysaccharides (Hu et al. 2017) exhibit in vitro anti-inflammatory activities, we demonstrated for the first time that Chenopodin also exhibits activity in in vivo models of inflammation.

3. Conclusion

The present study showed by N-terminal analysis that the vegetal protein isolated from C. quinoa is a Chenopodin and exhibits anti-inflammatory action decreasing paw edema and neutrophil recruitment induced by carrageenan in mice.
Disclosure statement
The authors declare no conflict of interest.

Funding
This work was supported by the National Council for Scientific and Technological Development (CNPq) under number 303307/2018-8; and Minas Gerais Research Foundation (FAPEMIG) under number APQ-03120-16. We are grateful to Coordination for the Improvement of Higher Education Personnel (CAPES – Finance code 001).

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