Free Rad. Res., Vol. 33, pp. 661-665
Reprints available directly from the publisher
Photocopying permitted by license only

Activity of Artichoke Leaf Extract on Reactive Oxygen Species in Human Leukocytes

FRANCISCO PÉREZ-GARCÍA, TOMÀS ADZET and SALVADOR CAÑIGUERAL*

Unitat de Farmacologia i Farmacognòsia. Facultat de Farmàcia. Universitat de Barcelona. Av. Diagonal, 643. E-08028 Barcelona, Spain

Accepted for publication by Prof. C. Rice-Evans

(Received 01 March 1999; In revised form 25 April 2000)

Artichoke leaf extract was studied in human leukocytes for activity against oxidative stress using flow cytometry and dichlorofluorescin diacetate as a fluorescence probe. It produces a concentration-dependent inhibition of oxidative stress when cells are stimulated with agents that generate reactive oxygen species (ROS): hydrogen peroxide, phorbol-12-myristate-13-acetate (PMA), and N-formyl-methionyl-leucyl-phenylalanine (FMLP). Cynarin, caffeic acid, chlorogenic acid, and luteolin, constituents of artichoke leaf extract, also show a concentration-dependent inhibitory activity in the above models, contributing to the antioxidant activity of the extract in human neutrophils.

Keywords: Neutrophils; flow cytometry; reactive oxygen species; artichoke; *Cynara scolymus*; active constituents

INTRODUCTION

The traditional use of artichoke (*Cynara scolymus* L.) leaf extract in medicine is based mainly upon its strong antidyspeptic actions[1,2]. Apart from that, it also reduces cholesterol biosynthesis in primary cultured hepatocytes at doses between 0.7 μg/ml and 100 μg/ml. Screening of several known constituents of artichoke extract revealed that luteolin was mainly responsible for this inhibition and chlorogenic acid showed also some activity, whereas caffeic acid, cynarin and other dicaffeoylquinic acids were without significant influence [3,4]. Previous investigations showed strong antioxidant effects for artichoke leaf extract on several test systems. Artichoke leaf extract reduced CCl_4 radical hepatotoxicity in rats [5]. Only cynarin and, to a lesser extent, caffeic acid showed cytoprotective action [6] on CCl_4 toxicity in isolated rat hepatocytes. Extracts from leaves of artichoke showed antioxidant action against hydroperoxide-induced oxidative stress in cultured rat hepatocytes in concentrations down to 1 μg/ml. Chlorogenic acid and cynarin accounted for part of this activity [7].

In addition, an antioxidant effect was also observed in the form of a reduction of LDL oxidation. Artichoke extract prevented LDL oxidation induced by copper, in part due to the flavonoid luteolin [8]. Moreover, caffeic and chlorogenic acid showed antioxidant activities by increasing the resistance of LDL to peroxidation, protecting LDL cholesterol from oxidation, and preventing the oxidative modification of the LDL apoprotein B100, probably through a peroxyl radical scavenging action [8]. Caffeic acid

* Corresponding author Salvador Cañigueral, Unitat de Farmacologia i Farmacognòsia. Facultat de Farmàcia. Universitat de Barcelona. Av. Diagonal, 643. E-08028 Barcelona, Spain. Phone: 34–93–4024531. Fax: 34–93–4035982. E-mail: caniguer@farmacia.far.ub.es

seems to form a transient chelating complex with cupric ions, coupled with its free radical scavenging properties, accounts for its inhibitory activity [9].

For the extract of artichoke leaves, pharmacokinetic data are not yet available, but pharmacological effects, including clinical data, suggest enough bioavailability for the active constituents of the extract. Evidence for the bioavailability of hydroxycinnamates and flavonoids is growing [10–12].

Considering the results available on pharmacological activities of artichoke extract, the next step was to investigate its actions on appropriate human blood cell systems, since epidemiological studies based on assessment of intake and clinical endpoints have shown a role for polyphenolic compounds in disease prevention and health protection [13–14]. Thus, the main objective of the present work was to study the activity of artichoke leaf extract on oxidative stress in human blood leukocytes stimulated by several ROS-provoking agents: hydrogen peroxide, phorbol-12-myristate-13-acetate (PMA) and N-formyl-methionyl-leucyl-phenylalanine (FMLP). Whether cynarin, caffeic acid, chlorogenic acid and luteolin, constituents of artichoke leaf extract, could be responsible, in part, for such activity was also investigated.

MATERIALS AND METHODS

Materials

Hanks Balanced Salt Solution (HBSS), phorbol-12-myristate-13-acetate (PMA), N-formyl-methionyl-leucyl-phenylalanine (FMLP), propidium iodide, caffeic acid, chlorogenic acid and luteolin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Hydrogen peroxide was obtained from Jansen (Geel, Belgium), 2′,7′-dichlorofluorescin diacetate from Serva (Heidelberg, Germany), and the components of the lysis solution were from Panreac (Montcada i Reixac, Spain). Cynarin was obtained from Extrasynthèse (Merignac, France). Human blood cells were obtained from healthy donors in the Hospital de la Vall d'Hebron (Barcelona, Spain) in the form of a buffy coat preparation. The flow cytometer used was an EPICS XL (Coulter, Miami, FL, USA).

Artichoke leaf extract

The artichoke leaf extract tested was Supra-Sern®, used in the drug Hepar SL Forte® (Sertürner Arzneimittel GmbH, Berlin, Germany), and the preparation consisted of 80% genuine artichoke leaf extract, 17% lactose, and 3% colloidal silicon dioxide. Content of caffeoylquinic acids was 1.5% (calculated as chlorogenic acid) and content of flavonoids was 0.5% (calculated as luteolin-7-O-glucoside).

Antioxidant assay

Flow cytometry was used for evaluating ROS in human neutrophils as described previously [15,16]. The method was adapted for the study of natural products [17]. 2′,7′-Dichlorofluorescin diacetate (DCFH-DA) was used as a fluorescence probe for ROS measurement. After DCFH-DA enters the cells it is deacetylated to dichlorofluorescin (DCFH) by a cellular esterase. Then, if ROS are present in the cells, they oxidize the DCFH to fluorescent 2′,7′-dichlorofluorescein (DCF). Cellular fluorescence is proportional to ROS production, and can be monitored by flow cytometry.

Enriched leukocytes were obtained from the buffy coat by hypoosmotic lysis and washing with HBSS. Leukocytes were incubated (37°C, 15 min) with the fluorescence probe DCFH-DA (10 μM), after which the cells were incubated (37 °C, 5 min) with the stimulant (H_2O_2, 100 μM; PMA, 10 μM or FMLP, 10 μM) in the presence of the blank or tested materials. Artichoke leaf extract was tested at concentrations between 100 μg/ml and

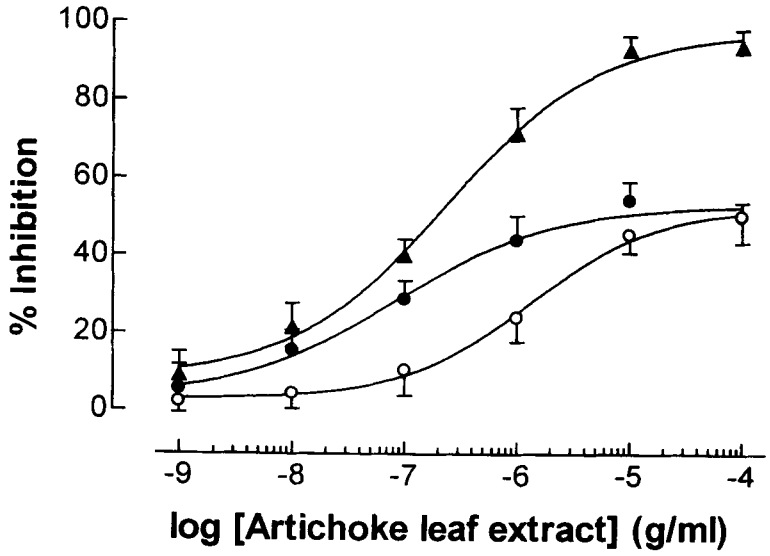

FIGURE 1 Inhibitory activity of artichoke leaf extract on ROS production in human neutrophils stimulated with H_2O_2 (○), PMA (▲) and FMLP (●)

1 ng/ml using a blank with lactose and colloidal silicon dioxide in HBSS. Cynarin, caffeic acid, chlorogenic acid and luteolin were assayed over a micromolar range, using HBSS as the blank. Finally, preparations were analyzed by flow cytometry: the leukocyte subpopulations were discriminated and the fluorescence of 10.000 neutrophils from each sample was measured.

All measurements were repeated using leukocyte samples from 10 different volunteers. Viability was measured using propidium iodide in the flow cytometer during the experiment: it was always more than 95%.

Concentration-response curves were analyzed according to a sigmoidal model of variable slope (Graph Pad Prism, San Diego, USA).

RESULTS AND DISCUSSION

Both artichoke leaf extract (Figure 1) and the pure constituents cynarin, caffeic acid, chlorogenic acid and luteolin (Figure 2) reduce ROS production induced by the three stimulants H_2O_2, PMA and FMLP in a concentration-dependent manner.

H_2O_2 is a key compound in free radical metabolism since it can be transformed into other dangerous ROS in the cell. PMA is a phorbol ester that acts as a tumour promoter and FMLP is a chemotactic peptide released from bacteria during infection. Both stimulate ROS generation by different mechanisms.

When H_2O_2 is used as stimulant, artichoke leaf extract produces ca. 50% inhibition at concentrations between 10 µg/ml (45.4 %) and 100 µg/ml (50.3 %). The pure constituents assayed show the same level of inhibition at lower concentrations, between 3.5 µg/ml and 9.0 µg/ml: chlorogenic acid at 3.5 µg/ml (66.1 % inhibition), cynarin at 5.2 µg/ml (55.2 %), caffeic acid at 5.7 µg/ml (55.9 %) and luteolin at 9.0 µg/ml (51.6 %). Thus, these compounds give a clear contribution to the antioxidant activity of the artichoke leaf extract on human leukocytes stimulated with hydrogen peroxide.

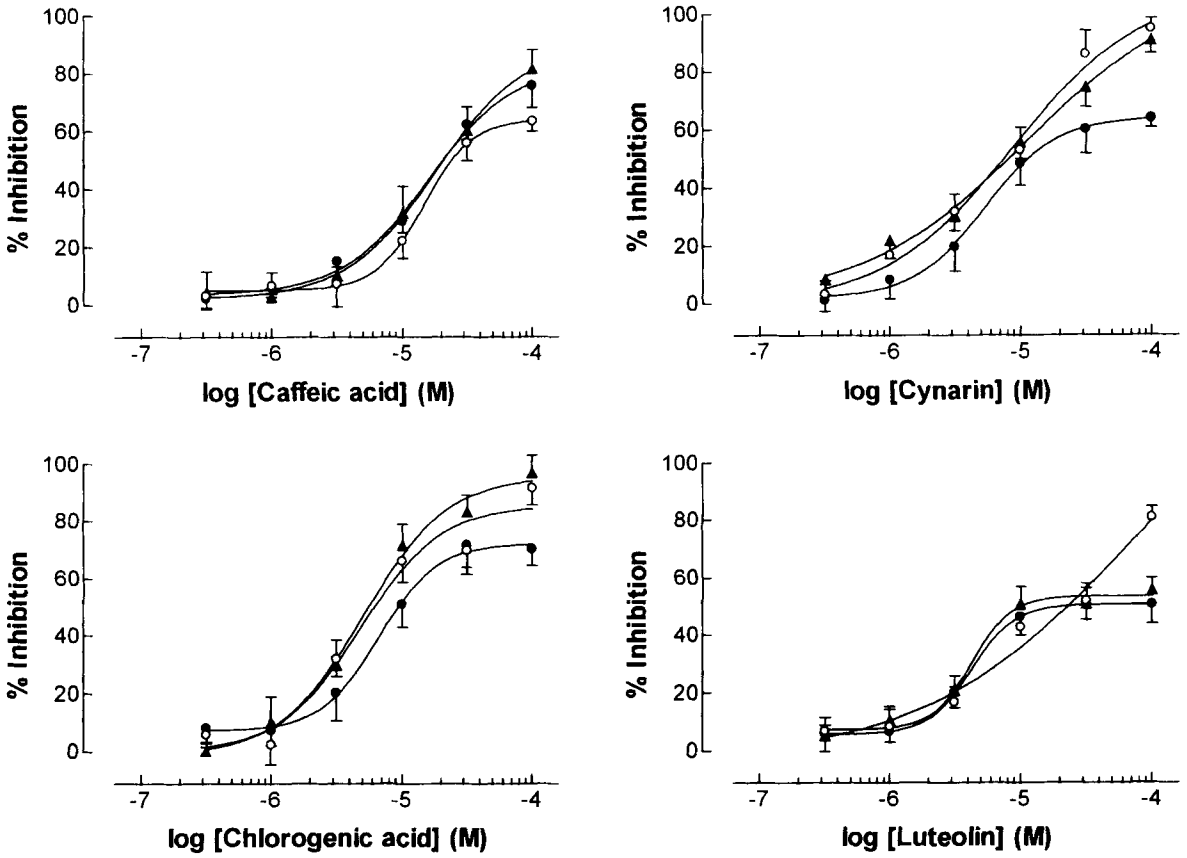

FIGURE 2 Inhibition of oxidative stress by constituents of the artichoke leaf extract. Activity of caffeic acid, chlorogenic acid, cynarin and luteolin on ROS production in human neutrophils stimulated with H_2O_2 (o), PMA (▲) and FMLP (●)

Concerning ROS production of neutrophils stimulated by FMLP, similar responses at the same concentrations (3.5–9.0 µg/ml) of the pure constituents than those found with H_2O_2 as stimulant were observed. Nevertheless, artichoke leaf extract inhibits from 44 % to 54 % at concentrations starting from 1 µg/ml. This indicates that chlorogenic acid, cynarin, caffeic acid and luteolin have only a moderate contribution to the antioxidant activity of the extract when the stimulant is FMLP.

Artichoke leaf extract shows strong inhibitory activity on ROS production induced by PMA (IC_{50} of 0.23 µg/ml). The pure constituents assayed have even less activity than the extract. This indicates that constituents other than chlorogenic acid, cynarin, caffeic acid and luteolin are the main components responsible of the antioxidant activity of the extract when the stimulant is PMA.

In summary, the investigated artichoke leaf extract shows relevant antioxidant properties in human neutrophils. The data correspond with the results obtained in other test systems, particularly in rat and human liver cells[6,7]. Furthermore, artichoke extract expresses its antioxidant action by a dual mechanism, both as a radical scavenger and as an inhibitor of PMA-induced radical generation.

Caffeic acid, chlorogenic acid, cynarin and luteolin have been confirmed to have a relevant contribution to the antioxidant activity of artichoke leaf extract in human neutrophils, specifically concerning the scavenging action. This is in accordance with recent literatura data [19–21]. In addition, results suggest that other constituents of the extract may participate in its antioxidant activity; specifically in neutrophil stimulated with PMA, where the polyphenols tested seem not to be responsible for the antioxidant effect of the extract.

Acknowledgements

The authors are grateful to Sertürner Arzneimittel GmbH (Berlin, Germany) for financial support through the Foundation Bosch i Gimpera of the University of Barcelona. The authors acknowledge the Scientific Technical Services of the University of Barcelona, and in particular those of Jaume Comas and Rosario González.

References

[1] K. Kraft (1997). Artichoke leaf extract – Recent findings reflecting effects on lipid metabolism, liver and gastrointestinal tracts. *Phytomedicine*, **4**, 369–378.

[2] R. Kirchhoff, Ch. Beckers, G. Kirchhoff, H. Trinczek-Gärtner, O. Petrowicz and H.-J. Reimann (1994). Increase in choleresis by means of artichoke extract. Results of a randomised placebo-controlled double-blind study. *Phytomedicine*, **1**, 107–115.

[3] R. Gebhardt (1997). Inhibition of hepatic cholesterol biosynthesis by artichoke leaf extracts is mainly due to luteolin. *Cell Biology and Toxicology* (abstract) **13**, 58.

[4] R. Gebhardt (1998). Inhibition of cholesterol biosynthesis in primary cultured rat hepatocytes by artichoke (*Cynara scolymus* L.) extracts. *Journal of Pharmacology and Experimental Therapeutics*, **286**, 1122–1128.

[5] T. Adzet, J. Camarasa and J.C. Laguna (1987). Hepatoprotective activity of polyphenolic compounds from *Cynara scolymus* against CCl4 toxicity in isolated rat hepatocytes. *Journal of Natural Products*, **50**, 612–617.

[6] R. Gebhardt (1997). Antioxidative and protective properties of extracts from leaves of the artichoke (*Cynara scolymus* L.) against hydroperoxide-induced oxidative stress in cultured rat hepatocytes. *Toxicology and Applied Pharmacology*, **144**, 279–286.

[7] R. Gebhardt and M. Fausel (1997). Antioxidant and hepatoprotective effects of artichoke extracts and constituents in cultured rat hepatocytes. *Toxicology in Vitro*, **11**, 669–672.

[8] J.E. Brown and C.A. Rice-Evans (1998). Luteolin-rich artichoke extract protects low density lipoprotein from oxidation in vitro. *Free Radical Research*, **29**, 247–255.

[9] M. Nardini, M. D'Aquino, G. Tomassi, V. Gentili, M. Di Felice, C. Scaccini. (1995) Inhibition of human low-density lipoprotein by caffeic acid and other hydroxycinnamic acid derivatives. *Free Radical Biology and Medicine* **19**, 541–552.

[10] L.C. Bourne and C.A. Rice Evans (1998). Urinary detection of hydroxycinnamates and flavonoids in humans after high dietary intake of fruit. *Free Radical Research* **28**, 429–438.

[11] J.P.E. Spencer, G. Chowrimootoo, R. Choudhury, E.S. Debnam, S.K. Srai, C. Rice-Evans (1999) The small intestine can both absorb and glucuronidate luminal flavonoids. *FEBS Letters* **458**, 224–230.

[12] R. Choudhury, S.K. Srai, E. Debnan and C.A. Rice-Evans (1999) Urinary excretion of hydroxycinnamates and flavonoids after oral and intravenous administration. *Free Radical Biology and Medicine* **27**, 278–286.

[13] M.G.L. Hertog, E.J.M. Feskens, P.C.H. Hollman, M.B. Katan and D. Kromhout (1993) Dietary flavonoids, antioxidant vitamins and coronary heart disease. The Zutphen eldery study. *The Lancet* **342**, 1007–1011.

[14] P. Knecht, R. Jarvinen, A. Reunanen and J. Maatela (1996) Flavonoid intake and coronary mortality in Finland: a cohort study. *British Medical Journal* **312**, 478–481.

[15] D.A. Bass, W. Parce, L.R. Dechatelet, P. Szejda, M.C. Seeds and M. Thomas (1983) Flow cytometric studies of oxidative product formation by neutrophils: a graded response to membrane stimulation. *Journal of Immunology*, **130**, 1910–1917.

[16] A. Imrich and L. Kobzik (1998). Flow cytometric analysis of macrophage oxidative metabolism using DCFH. *Methods in Molecular Biology*, **91**, 97–108.

[17] F. Pérez-García, E. Marín, S. Cañigueral and T. Adzet (1996). Anti-inflammatory action of *Pluchea sagittalis*. Involvement of an antioxidant mechanism. *Life Sciences* **59**, 2033–2040.

[18] C. Castelluccio, G. Paganga, N. Melikian, G.P. Bolwell, J. Pridham, J. Sampson, C. Rice Evans (1995). Antioxidant potential of intermediates in phenyl propanoid metabolism in higher plants. *FEBS Letters* **368**, 188–192.

[19] C.A. Rice-Evans, N.J. Miller, P.G. Bolwell, P.M. Bramley and J.B. Pridham (1995) The relative antioxidant activities of plant derived polyphenolic flavonoids. *Free Radical Research* **22**, 375–383.

[20] C.A. Rice-Evans, N.J. Miller and G. Paganga (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine* **20**, 933–956.

[21] S. Foley, S. Navaratman, D.J. McGarvey, E.J. Land, T.G. Truscott and C.A. Rice-Evans (1999) Singlet oxygen quenching and the redox properties of hydroxycinnamic acids. *Free Radical Biology and Medicine* **26**, 1202–1208.