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Indicaxanthin from Cactus Pear Fruit Exerts Anti-Inflammatory Effects in Carrageenin-Induced Rat Pleurisy[1,2]

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Abstract

Nutritional research has shifted recently from alleviating nutrient deficiencies to chronic disease prevention. We investigated the activity of indicaxanthin, a bioavailable phytochemical of the betalain class from the edible fruit of *Opuntia ficus-indica* (L. Miller) in a rat model of acute inflammation. Rat pleurisy was achieved by injection of 0.2 mL of λ-carrageenin in the pleural cavity, and rats were killed 4, 24, and 48 h later; exudates were collected to analyze inflammatory parameters, such as nitric oxide (NO), prostaglandin E_2 (PGE_2), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α); cells recruited in pleura were analyzed for cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) expression, and nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) activation. Indicaxanthin (0.5, 1, or 2 μmol/kg), given orally before carrageenin, time- and dose-dependently, reduced the exudate volume (up to 70%) and the number of leukocytes recruited in the pleural cavity (up to 95%) at 24 h. Pretreatment with indicaxanthin at 2 μmol/kg inhibited the carrageenin-induced release of PGE_2 (91.4%), NO (67.7%), IL-1β (53.6%), and TNF-α (71.1%), and caused a decrease of *IL-1β* (34.5%), *TNF-α* (81.6%), *iNOS* (75.2%), and *COX2* (87.7%) mRNA, as well as iNOS (71.9%) and COX-2 (65.9%) protein expression, in the recruited leukocytes. Indicaxanthin inhibited time- and dose- dependently the activation of NF-κB, a key transcription factor in the whole inflammatory cascade. A pharmacokinetic study with a single 2 μmol/kg oral administration showed a maximum 0.22 ± 0.02 μmol/L (n = 15) plasma concentration of indicaxanthin, with a half-life of 1.15 ± 0.11 h. When considering the high bioavailability of indicaxanthin in humans, our findings suggest that this dietary pigment has the potential to improve health and prevent inflammation-based disorders. J. Nutr. 144: 185–192, 2014.

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Introduction

Age-related inflammation-based disorders, such as cancer and cardiovascular disease, are widely acknowledged to have a lower incidence among populations whose dietary habits include a large proportion of vegetal food, thus providing various non-nutrient secondary metabolites (phytochemicals) with purported beneficial activities (1,2). Thus, despite the wealth of published papers, the interest in investigating how these food components may contribute to preventing and/or delaying the development of such chronic processes is far from waning.

Inflammation is an adaptative response triggered by noxious stimuli and conditions, such as infection and tissue injury. At a very basic level, it involves recruitment of blood components, plasma, and leukocytes, mainly neutrophils [polymorphonuclear cells (PMNs)],[5] to the site of infection or injury. This process is coordinated by a large range of biochemical mediators and results in the inflammatory exudate (3). The acute phase of an inflammatory response successfully ends up in the elimination of the insult, followed by a resolution phase, mainly mediated by tissue-resident and recruited macrophages (MPs).

Reactive oxygen and nitrogen species are now acknowledged to exert a key role in maintaining normal cellular and tissue physiology but may start signaling pathways involved in the development of a wide range of inflammation-based degenerative pathologies, including cardiovascular and neurodegenerative diseases, cancer, and aging itself (4–8). Reactive oxygen and nitrogen species play a crucial role during the acute phase of the inflammatory response because they are *1)* released from PMNs and MPs to eliminate the pathogen at the site of inflammation, *2)* involved in the synthesis of proinflammatory mediators, such as PGs, NO, and peroxynitrite, through both enzymatic and nonenzymatic free radical-catalyzed reactions, and *3)* essential in the redox-dependent cell signaling of inflammatory cells (9). In this context, numerous phytochemicals with peculiar reducing properties that may effectively downregulate the inflammatory response have recently been the object of research. First considered "health

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[5] Abbreviations used: COX-2, cyclooxygenase-2; iNOS, inducible NO synthase; MC, monocyte; MP, macrophage; NOx, oxidation products from nitrite; PMN, polymorphonuclear cell.
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promoting" by virtue of their radical-scavenging activity and/or direct antioxidant effects on cellular biomolecules, such compounds are now believed capable of interfering with cell signal transduction by intercepting reactive species at the level of critical signaling pathways. In addition, interaction of these molecules with signaling enzymes, receptors, and transcription factors has emerged recently (10).

Indicaxanthin [(2*S*)-2,3-dihydro-4-[2-[(2*S*)-2α-carboxypyrrolidin-1-yl]ethenyl]pyridine-2α,6-dicarboxylic acid], a phytochemical with the chemical structure of betalamic acid, is a dietary pigment from cactus pear fruit (*Opuntia ficus-indica*, L. Miller). A number of recent in vitro studies showed that indicaxanthin is a reducing and amphipathic molecule capable of penetrating cells and membranes and counteracting oxidative damage (11–18). In addition, it has been shown to act as a signaling molecule, being able to modulate specific redox-dependent pathways in cultured endothelial cells, thus protecting them from dysfunction (19). Remarkably, indicaxanthin is highly bioavailable in humans (20) and does not appear to be metabolized either during digestion or in the liver (21). Investigation from our group provided evidence that the ingestion of a dietary-consistent amount of cactus pear fruit pulp (4 fruits, containing 28 mg of indicaxanthin) generates, in humans, a plasma concentration of 7 μmol/L (20), quite a high amount compared with most of the dietary polyphenol phytochemicals, such as flavonoids (22,23). In this study, we investigated the activity of indicaxanthin, orally given to rats at doses consistent with a reasonable dietary intake, in a model of acute inflammatory condition, i.e., carrageenin-induced rat pleurisy. This is widely accepted as a robust pharmacologic model of inflammatory disease, used to ascertain the anti-inflammatory potential of drugs (24). A number of molecular markers of inflammation have been measured to shed light on pathways and mechanistic aspects associated with the modulatory activity of indicaxanthin.

Materials and Methods

Reagents. λ-Carrageenin (1% wt:v) was dissolved in sterile PBS. Unless otherwise specified, all the other reagents were from Sigma-Aldrich.

Extraction and purification of indicaxanthin from cactus pear fruits. Indicaxanthin was isolated from cactus pear (*O. ficus-indica*) fruits (yellow cultivar). Briefly, the phytochemical was separated from a methanol extract of the pulp by liquid chromatography on Sephadex G-25 as reported previously (11). Fractions containing the pigment were submitted to cryodesiccation, purified according to Stintzing et al. (25) and suspended in PBS for the experiments.

Animals. Male Wistar rats (Harlan) weighing 175–200 g were used in all experiments. Rats were fed and had access to water ad libitum. The light cycle was automatically controlled (on at 0700, off at 1900), and the room temperature was thermostatically regulated to 22 ± 1°C. Before the experiments, rats were housed and acclimatized under these conditions for 3–4 d. Animal care was in accordance with Italian and European regulations on the protection of rats used for experimental and other scientific purposes.

Induction of pleurisy and indicaxanthin treatment. Induction of pleurisy was as described previously (26). Briefly, rats were slightly anesthetized, and 0.2 mL of 1% λ-carrageenin suspended in sterile saline solution was injected into the right pleural cavity (control). Rats were killed 4, 24, and 48 h later in an atmosphere of CO_2. The exudate was harvested by washing each pleural cavity with 2 mL of sterile saline containing 5 U/mL heparin. Any exudate with blood contamination was discarded. Each sample was centrifuged at 800 × *g* for 10 min, and the cell pellet was resuspended in saline. Total cell count was estimated after trypan blue staining using a Burker counting chamber, whereas differential cell count was determined in smears by May-Grunwald staining. In parallel, groups of rats received indicaxanthin (0.5, 1, or 2 μmol/kg) by oral gavage 30 min before carrageenin injection and every 8 h thereafter, up to 40 h, with control rats receiving saline alone. Rats were killed at 4, 24, and 48 h after carrageenin injection, and pleural exudates were collected and processed as described above. In some experiments, rats received a single oral administration of anti-inflammatory drug (3 mg/kg indometacin), 30 min before carrageenin injection (26). Pharmacokinetics of indicaxanthin were followed by measuring plasma concentration and urinary excretion of the molecule, after a single administration of 2 μmol/kg. Each experiment was performed three times with groups of 10 rats, with the exception of the pharmacokinetic measurements (five rats). Data from separate experiments were pooled.

Preparation of cell extracts. The inflammatory cell pellets were washed twice in PBS (ICN Biomedicals) and centrifuged at 800 × *g* for 5 min at 4°C. Whole-cell and nuclear extracts were prepared as described previously (26), aliquoted, and stored at −80°C. Protein concentration was determined by the Bio-Rad protein assay kit.

RT-PCR. Pleural cells were collected under sterile conditions, and total RNA was isolated using TRIzol Reagent (Invitrogen). Levels of interleukin-1β (*IL-1β*), tumor necrosis factor-α (*TNF-α*), inducible NO synthase (*iNOS*), and cyclooxygenase-2 (*COX2*) mRNA were evaluated by PCR amplification of reverse-transcribed mRNA as described previously (27). Parallel amplification of the rat housekeeping gene *β-actin* was performed as internal control. The sequences of the PCR primers were as follows: for *TNF-α*, 5′-GTTCTATGGCCCAGACCC-TCACA-3′ (sense) and 5′-TACCAGGGTTTGAGCTCAGC-3′ (antisense); for *IL-1β*, 5′-GACCTGTTCTTTGAGGCTGAC-3′ (sense) and 5′-TCCATCTTCTTCTTTGGGTATTGTT-3′ (antisense); for *iNOS*, 5′-GTGTTCCACCAGGAGATGTTG-3′ (sense) and 5′-CTCCTGCCCA-CTGAGTTCGTC-3′ (antisense); for *COX2*, 5′-TGTATGCTACCATC-TGGCTTCGG-3′ (sense) and 5′-GTTTGGAACAGTCGCTCGTCATC-3′ (antisense); and for *β-actin*, 5′-ATGAAGATCCTGACCGAGGCGT-3′ (sense) and 5′-AACGCAGCTCAGTAACAGTCCG-3′ (antisense). The PCR products were run on a 2% agarose gel and visualized by ethidium bromide staining.

Western blotting. Cell lysates were prepared as described previously (28), collected, and stored at −20°C until tested. Protein concentration was determined by Bradford assay according to the instructions of the manufacturer (Bio-Rad). Immunoblotting analysis of cyclooxygenase-2 (COX-2), iNOS, and β-actin proteins was performed as described previously (28) with polyclonal antibodies against COX-2, iNOS, or β-actin (Santa Cruz Biotechnology).

Electrophoretic mobility-shift assay. NF-κB/DNA binding activity was evaluated by electrophoretic mobility-shift assay in nuclear extracts as described previously (29). Briefly, 10 μg of protein from each sample was incubated for 20 min with ^{32}P-labeled, double-stranded NF-κB consensus binding-site oligonucleotides in 20 μL of binding reaction buffer. The specificity of the DNA/protein binding was determined by competition reaction in which a 50-fold molar excess of unlabeled wild-type, mutant, or AP-1 oligonucleotide was added to the binding reaction 10 min before addition of the radiolabeled probe. In supershift assay, antibodies reactive to p50, p65, or p50 + p65 proteins were added to the reaction mixture 30 min before the radiolabeled NF-κB probe. Nuclear protein–oligonucleotide complexes were analyzed by gel electrophoresis. Gels were dried and autoradiographed with intensifying screen at −80°C for 24 h. Quantitative evaluation of NF-κB/DNA complex formation was determined by densitometric analysis performed with a GS-700 imaging densitometer (Bio-Rad) and a data computer program (Molecular Analyst; IBM).

PGE_2, TNF-α, and IL-1β determination. Levels of PGE_2 in the pleural exudates were measured using a 96-well-based enzyme immunoassay kit from Cayman Chemicals (Inalco). The proinflammatory cytokines TNF-α and IL-1β were evaluated using an ELISA kit from eBioscience.

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Determination of oxidation products from nitrite. The amount of oxidation products from nitrite (NOx) in the inflammatory exudate was measured after reducing NO_3^- to NO_2^- by acid-washed cadmiun powder, NO_2^- (30), followed by Griess reaction and spectrophotometric measurement of NO_2^- (31).

Data handling and statistical analyses. Data are expressed as means ± SEMs. Comparisons were made using 1-factor ANOVA, followed by a Bonferroni test. Time-course comparisons, including time, dose, and dose × time in the model, were tested by 2-factor ANOVA. Differences between doses at each time level were assessed by a Bonferroni test (Instat-3 statistical software; GraphPad Software). Comparisons between effectiveness of indicaxanthin and indomethacin were made by Student's t test.

Results

Indicaxanthin counteracts the acute phase of carrageenin-induced rat pleurisy. The pleural cavity of untreated rats comparable with those used in this work contains no exudate and physiologic amounts of leukocytes, predominantly mononuclear (>95%) (32). Injection of 0.2 mL of λ-carrageenin into the pleural cavity of rats (control) produced a clear time-dependent inflammatory response, evident as an increase of both the exudate volume and the number of leukocytes migrated into the pleural cavity. In line with our previously reported data (32), an increase of these parameters was observed at 4 h, whereas the acute phase of inflammation peaked at 24 h. The inflammatory response was shut down at 48 h (**Fig. 1***A*, *B*). Differential cell count of leukocytes migrated into the pleural cavity showed that PMNs dominated the early phase (4 h) of the reaction [91% of PMNs and 9% of monocytes (MCs)] and were replaced by MCs (29% of PMNs and 71% of MCs) at 48 h (data not shown).

With respect to control rats, oral administration of indicaxanthin (2 μmol/kg) 30 min before carrageenin injection and then for 48 h, at 8 h time intervals, significantly decreased the inflammatory response at all the time points considered (4, 24, and 48 h), with inhibition of both the exudate volume and the total leukocyte number. The inhibitory effect at the peak of the inflammatory response accounted for >67% and 90% for the exudate volume and leukocyte number, respectively, and appeared greater than the inhibitory effects observed at 4 and 48 h (Fig. 1*A*, *B*). Carrageenin-treated rats were administered 0.5–2.0 μmol/kg indicaxanthin (**Fig. 2**). With respect to control, both the exudate volume (Fig. 2*A*) and the pleural leukocyte number (Fig. 2*B*) decreased significantly at 0.5 μmol/kg, with the maximum effect at 1 μmol/kg (70% and 95% inhibition of the exudate volume and pleural cell number, respectively). An additional decrease at 2.0 μmol/kg was not significant. In parallel experiments, carrageenin-treated rats were given indometacin as a reference anti-inflammatory drug (26), and the effect was compared with rats administered 1 μmol/kg indicaxanthin. Indicaxanthin exhibited the same effectiveness as indometacin at reducing the exudate volume (n = 30; P = 0.25, Student's t test) and was even more effective at inhibiting the cell recruitment into the pleural cavity (n = 30; P < 0.0001, Student's t test).

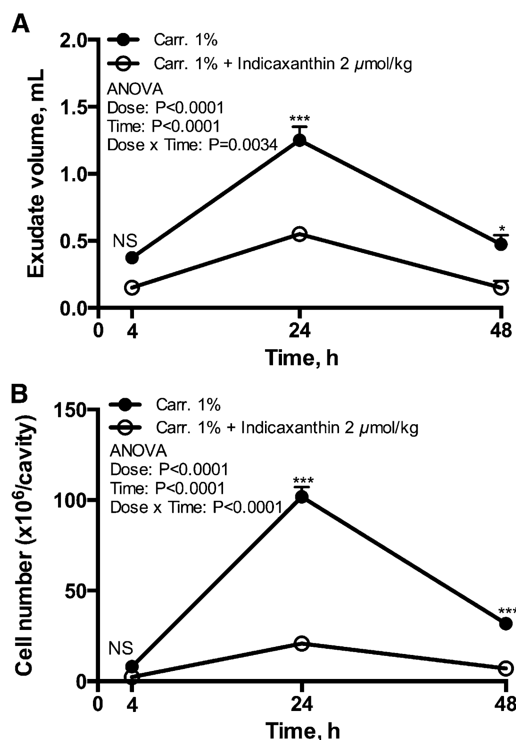

FIGURE 1 Effect of indicaxanthin on the time course of exudate formation (*A*) and leukocyte infiltration (*B*) in carrageenin-induced rat pleurisy. Values are means ± SEMs, n = 30 (10 rats in 3 separate experiments). Dose differences at each time level were assessed by a Bonferroni test. Asterisks indicate that means at a time differ: *P = 0.0169; ***P < 0.0001; NS indicates P > 0.05. Carr, carrageenin.

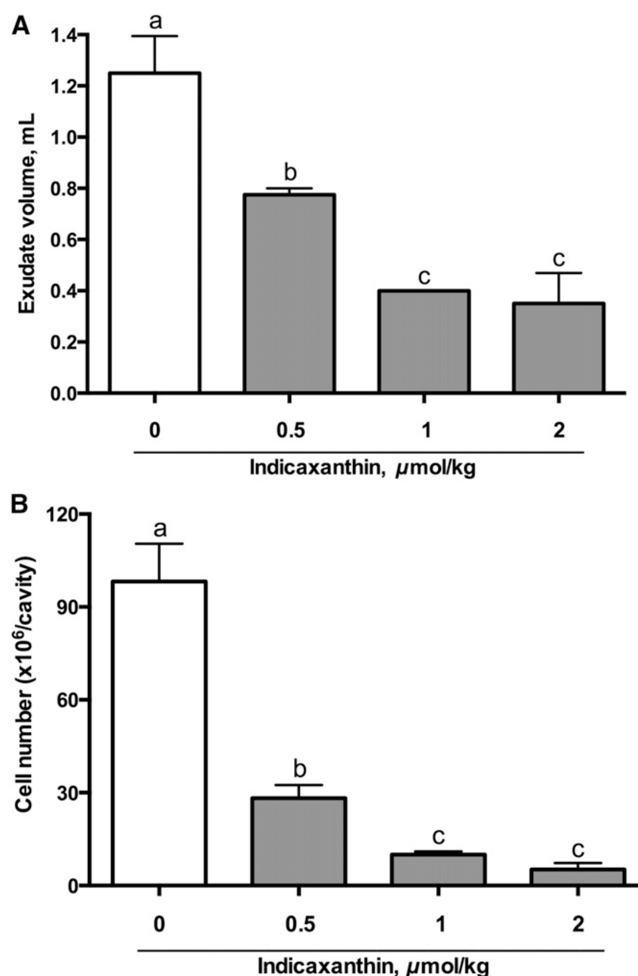

FIGURE 2 Exudate volume (*A*) and leukocyte infiltration (*B*) at 24 h in carrageenin-treated rats administered 0–2 μmol/kg indicaxanthin. Values are means ± SEMs, n = 30 (10 rats in 3 separate experiments). Labeled means without a common letter differ: P < 0.05.

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Indicaxanthin inhibits the release of proinflammatory mediators during carrageenin-induced rat pleurisy. The release into the pleural cavity of a panel of proinflammatory soluble mediators supporting the acute phase of the inflammatory response (i.e., PGE_2, NOx, IL-1β, and TNF-α) was then evaluated in either the absence or presence of indicaxanthin supplementation. The injection of 0.2 mL of λ-carrageenin into the pleural cavity of rats caused a release of PGE_2, NOx, IL-1β, and TNF-α at 24 h. Inflammatory mediators are not detectable in rats before injection (32). Oral administration of indicaxanthin at 0.5, 1, and 2 μmol/kg dose dependently decreased the release of all mediators (**Fig. 3**).

Indicaxanthin inhibits the expression of proinflammatory genes in carrageenin-induced rat pleurisy. Proinflammatory genes are not expressed in rats before carrageenin injection (32). The level of *IL-1β*, *TNF-α*, *iNOS*, and *COX2* mRNA at 4 h (**Fig. 4***A–E*) and iNOS and COX-2 protein expression at 24 h (Fig. 4*F–H*) were evaluated in the cells recruited in the pleural cavity of rats injected with carrageenin that did or did not receive indicaxanthin. Oral administration of indicaxanthin at 0.5, 1, and 2 μmol/kg dose-dependently decreased all the carrageenin-induced inflammatory parameters, with the exception of *IL-1β* (Fig. 4*B*).

Indicaxanthin inhibits NF-κB activation in carrageenin-induced rat pleurisy. The inflammatory response is under control of several transcription factors, including NF-κB (33). The mechanisms underlying the anti-inflammatory effects of indicaxanthin were investigated by evaluating the effect of the phytochemical on the activation state of NF-κB. The band of NF-κB/DNA complex was evident 4 h after carrageenin injection, peaked at 24 h, and then decreased but was still detectable 48 h later (**Fig. 5***A*). Oral administration of indicaxanthin (2 μmol/kg) decreased the activation state of NF-κB at 4 and 24 h, with no effect at 48 h (Fig. 5*A*). The effect at 24 h was dose-dependent in the indicaxanthin range of 0.5–2.0 μmol/kg. (Fig. 5*B*).

Pharmacokinetics of indicaxanthin in rats. The pharmacokinetic profile of indicaxanthin was assessed by evaluating plasma kinetics and urinary excretion of the pigment after a single oral administration of 2 μmol/kg. The phytochemical was detectable after 45 min, and a 0.2 μmol/L plasma peak concentration was reached 2 h after ingestion (**Fig. 6**). The compound disappeared from plasma after 4 h. Log transformation of the plasma concentrations during the period 2–4 h after ingestion indicated that the disposal of indicaxanthin followed first-order kinetics and had a calculated half-life of 1.15 $\pm$ 0.11 h. Urine samples were collected over 12 h, and the total amount of indicaxanthin excreted was 21 $\pm$ 1.2% of the administered dose. Because indicaxanthin is not metabolized at either the intestinal or liver level (20,21), conjugation products were not measured in urine.

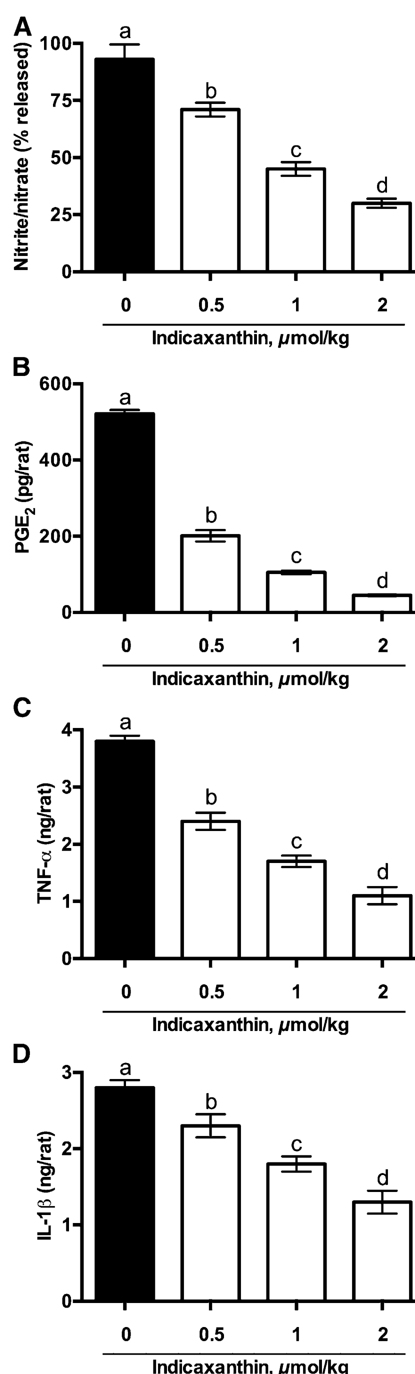

FIGURE 3 Release of proinflammatory mediators nitrite/nitrate (*A*), PGE_2 (*B*), TNF-α (*C*), and IL-1β (*D*) at 24 h in carrageenin-treated rats administered 0–2 μmol/kg indicaxanthin. Values are means $\pm$ SEMs, n = 30 (10 rats in 3 separate experiments). Labeled means without a common letter differ: $P < 0.05$.

Discussion

Dietary phytochemicals are continuously investigated to understand the basis of protective activity of fruits and vegetables against chronic inflammation-based disorders and to discover any nutraceutical potential of specific compounds. With this study, to the best of our knowledge, the health-promoting effects of indicaxanthin have been explored for the first time in vivo in an animal model of acute inflammation. Our data show that this betalain has a remarkable anti-inflammatory activity and therapeutic potential.

Food phytochemicals associated with health benefits include glucosynolates, carotenoids, and phytosterols and a large number of polyphenols. The latter, in particular, because of their chemistry, have been considered ideal candidates to regulate and/or interfere with the redox-dependent signaling processes underlying the inflammatory response (1). However, a key issue to decide about the real beneficial effects of dietary phytochemicals on human health is their bioavailability, which must be referred not only to the tested compounds but also to their metabolic products (23,34). Anti-inflammatory activity and effects exhibited by plant

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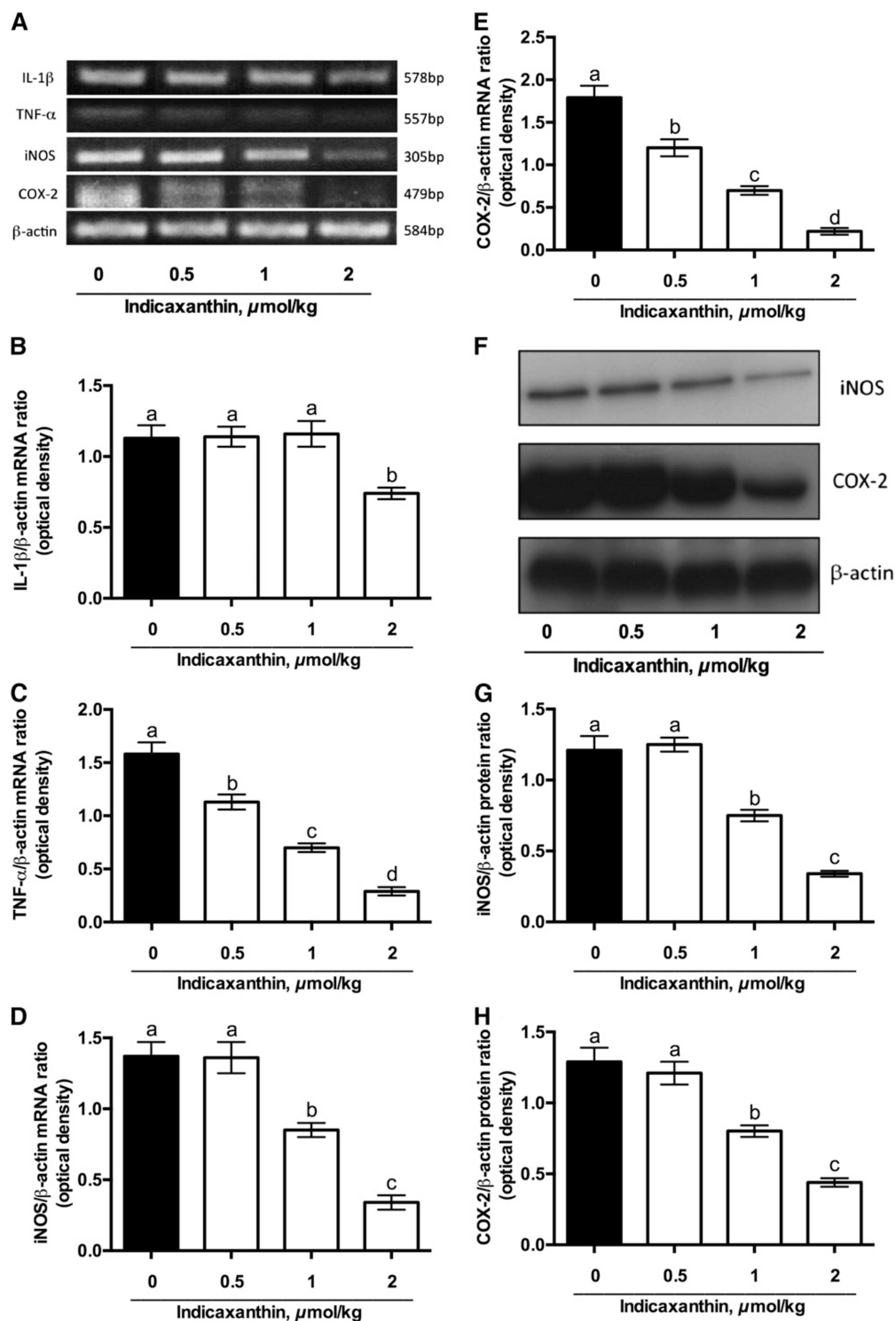

FIGURE 4 *IL-1β*, *TNF-α*, inducible NO synthase (*iNOS*), and cyclooxygenase-2 (*COX2*) mRNA expression (*A–E*) and iNOS and COX-2 protein levels (*F–H*) in carrageenin-treated rats administered 0–2 mmol/kg indicaxanthin. Immunoblots are representative images of three experiments with comparable results. Values are means ± SEMs, $n = 30$ (10 rats in 3 separate experiments). Labeled means without a common letter differ: $P < 0.05$. *COX2*, cyclooxygenase-2; *iNOS*, inducible NO synthase.

polyphenols in vitro are frequently diminished or even lost in vivo because of incomplete absorption and first-pass metabolism (23,35). In addition, some dietary phytochemicals reported to modulate the inflammatory response are effective only when intraperitoneally administered (36,37) or given orally at doses too high to be of nutritional interest.

A number of studies from our and other laboratories showed that 2 major dietary betalain pigments, such as betanin and indicaxanthin, are bioavailable in humans from various food sources (20,38,39) and are not modified to be absorbed (20,40). Indicaxanthin, in particular, measured in human plasma after consumption of dietary amounts of cactus pear fruit pulp, reached a high micromolar peak at 3 h, with a clearance with first-order kinetics in the following 5 h (20), and an excreted amount representing ~70% of that ingested with food (20). We demonstrate here that even pure indicaxanthin, given orally to rats at amounts comparable with its intake with a reasonable amount of food (20), is absorbed and is recovered unmodified in plasma. There also is evidence that, at least in rats, the pure molecule, even deprived of its food matrix, is resistant to the gastrointestinal digestion process and is bioavailable. This is in line with previous findings from simulated gastrointestinal digestion showing that >70% of indicaxanthin, either from food or as a pure compound, was recovered in the postintestinal bioaccessible fraction (41), further indicating the remarkable digestive stability of this molecule.

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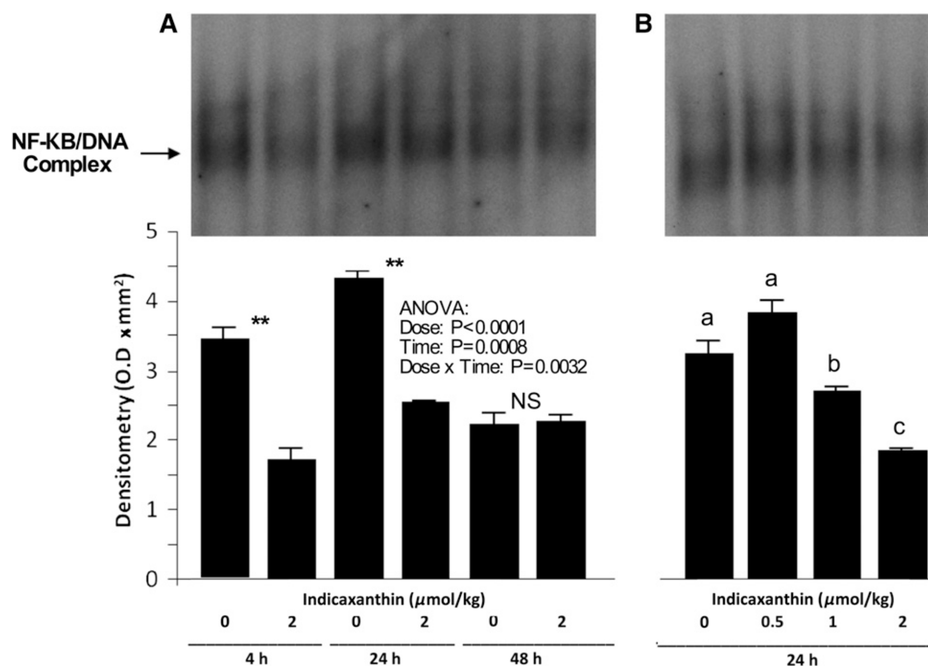

FIGURE 5 NF-κB/DNA binding activity in carrageenin-treated rats administered 0–2 μmol/kg indicaxanthin. Kinetic profile (*A*) and dose dependency (*B*). Electrophoretic mobility-shift assay image is representative of three experiments with comparable results. Values are means ± SEMs, n = 30 (10 rats in 3 separate experiments). In *A*, asterisks indicate that means at a time differ (by a Bonferroni test): $^{**}P$ = 0.0022; in *B*, labeled means without a common letter differ: $P < 0.05$; NS indicates $P > 0.05$.

Carrageenin causes a reproducible inflammatory reaction and is considered a standard chemical for examining effects of anti-inflammatory drugs (24,42). It has been used in animal models of colon inflammation, but also of extracolonic sites, including pleural space and peritoneum. In our carrageenin-induced rat pleurisy, we observed that indicaxanthin, administered orally 30 min before the inflammatory insult and subsequently at 8 h intervals, suppressed dose-dependently the onset of the acute phase of the inflammatory response by decreasing both the number of inflammatory cells recruited into the pleural cavity and the volume of exudate at 24 h. An almost total prevention (95%) of the acute response at 24 h was observed at the highest amount (2 μmol/kg). This anti-inflammatory effect was associated with a reduction of release into the pleural cavity of a number of proinflammatory mediators, such as PGE_2, and oxidation products from nitrite, from the activity of COX and iNOS, respectively, as well as the cytokines IL-1β and TNF-α.

Onset and progression of inflammatory response are complex and dynamic processes involving pathophysiologic mechanisms with sequential recruitment of different cell types and phenotypical and functional cell evolution, and a number of biochemical reactions. In response to noxious stimuli, tissue-resident MPs start the response with secretion of TNF-α and IL-1β, broad-spectrum cytokines that stimulate neutrophils, fibroblasts, and endothelial cells (32). The response of the latter mainly consists of recruiting other immune cells to the site of inflammation, thus amplifying and perpetuating the inflammatory reaction. In our carrageenin-inflamed animal model, oral administration of indicaxanthin caused a rapid decrease of *IL-1β*, *TNF-α*, *iNOS*, and *COX2* mRNA in the leukocytes recruited in the pleural cavity at the early phase of the inflammatory response, followed by the inhibition of iNOS and COX-2 protein expression at the acute phase, indicating an effect at the transcriptional level and possibly suggesting intervention of the molecule on inflammatory pathways driving the evolution of the inflammatory response. Our recent in vitro studies demonstrate that indicaxanthin can elicit formation of anti-inflammatory prostanoids, such as PGJ_2 in cultured rat MPs (M. Allegra, F. D'Acquisto, L. Tesoriere, A. Attanzio, M. A. Livrea, unpublished data). The observed effects of indicaxanthin were significantly greater on the cells at the acute phase. However, our experimental design precludes whether a specific cell, whether PMN or monocyte/MC, is targeted by indicaxanthin because of the dynamic and continuous cell transformation from one phenotype to another.

Distribution of absorbed dietary compounds in tissues or cells is essential to their eventual activity. Our previous human study showed that absorbed indicaxanthin can distribute within red blood cells (20). The present findings show activity of the molecule at the level of leukocytes recruited in the pleural cavity, indicating that indicaxanthin can enter in other cells.

At a molecular level, the inflammatory activity of carrageenin has been shown to go through activation of the pleiotropic NF-κB (43), a key factor of the inflammatory cascade necessary to activate transcription of proinflammatory genes. Consensus-binding sequences for NF-κB have been identified in the promoter regions of several genes implicated in the pathogenesis of acute and chronic inflammation, such as *TNF-α*, *IL-1β*, *COX2*, and *iNOS*, which makes NF-κB a main target for a number of potential anti-inflammatory molecules (44) and its inhibition a very promising weapon to fight inflammation-based disorders

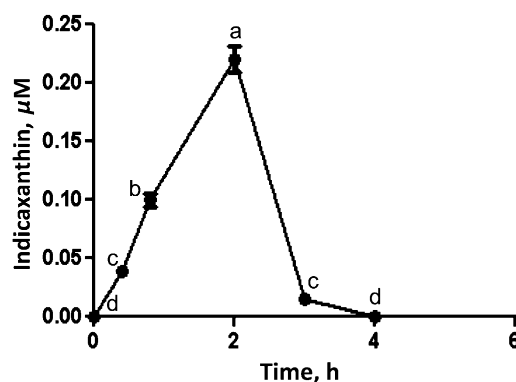

FIGURE 6 Kinetics of plasma indicaxanthin in rats after ingestion of 2 μmol/kg. Values are means ± SEMs, n = 15 (five rats in 3 separate experiments). Labeled means without a common letter differ: $P < 0.05$.

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(45). In this context, the observation that anti-inflammatory effects of indicaxanthin can be related, at least in part, to its ability to reduce NF-κB activation appears of a great relevance to consider its potential as health-promoting substance. In conclusion, to the best of our knowledge, this study demonstrates the remarkable pharmacologic effects exerted by indicaxanthin in an in vivo model of the inflammatory condition and establishes for the first time a health-promoting potential for indicaxanthin administered orally at nutritionally relevant doses.

Acknowledgments

M.A. and A.I. designed the research; M.A., M.T., E.P., and L.T. conducted the research; M.A., A.I., and M.A.L. wrote the paper; and M.A.L. had primary responsibility for the final content. All authors read and approved the final manuscript.

Literature Cited

1. Virgili F, Marino M. Regulation of cellular signals from nutritional molecules: a specific role for phytochemicals, beyond antioxidant activity. Free Radic Biol Med. 2008;45:1205–16.
2. Halliwell B, Rafter J, Jenner A. Health promotion by flavonoids, tocopherols, tocotrienols, and other phenols: direct or indirect effects? Antioxidant or not? Am J Clin Nutr. 2005;81:268S–76S.
3. Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008;454:428–35.
4. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39:44–84.
5. Koutsilieri E, Scheller C, Grunblatt E, Nara K, Li J, Riederer P. Free radicals in Parkinson's disease. J Neurol. 2002;249(suppl 2):II1–5.
6. Closa D, Folch-Puy E. Oxygen free radicals and the systemic inflammatory response. IUBMB Life. 2004;56:185–91.
7. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact. 2006;160:1–40.
8. Wickens AP. Ageing and the free radical theory. Respir Physiol. 2001; 128:379–91.
9. Basu S. Bioactive eicosanoids: role of prostaglandin F(2alpha) and F-isoprostanes in inflammation and oxidative stress related pathology. Mol Cells. 30:383–91.
10. Leonarduzzi G, Sottero B, Poli G. Targeting tissue oxidative damage by means of cell signaling modulators: the antioxidant concept revisited. Pharmacol Ther. 128:336–74.
11. Butera D, Tesoriere L, Di Gaudio F, Bongiorno A, Allegra M, Pintaudi AM, Kohen R, Livrea MA. Antioxidant activities of sicilian prickly pear (*Opuntia ficus indica*) fruit extracts and reducing properties of its betalains: betanin and indicaxanthin. J Agric Food Chem. 2002;50: 6895–901.
12. Tesoriere L, Butera D, D'Arpa D, Di Gaudio F, Allegra M, Gentile C, Livrea MA. Increased resistance to oxidation of betalain-enriched human low density lipoproteins. Free Radic Res. 2003;37:689–96.
13. Allegra M, Furtmuller PG, Jantschko W, Zederbauer M, Tesoriere L, Livrea MA, Obinger C. Mechanism of interaction of betanin and indicaxanthin with human myeloperoxidase and hypochlorous acid. Biochem Biophys Res Commun. 2005;332:837–44.
14. Tesoriere L, Allegra M, Butera D, Gentile C, Livrea MA. Cytoprotective effects of the antioxidant phytochemical indicaxanthin in beta-thalassemia red blood cells. Free Radic Res. 2006;40:753–61.
15. Tesoriere L, Allegra M, Butera D, Gentile C, Livrea MA. Kinetics of the lipoperoxyl radical-scavenging activity of indicaxanthin in solution and unilamellar liposomes. Free Radic Res. 2007;41:226–33.
16. Tesoriere L, Butera D, Allegra M, Fazzari M, Livrea MA. Distribution of betalain pigments in red blood cells after consumption of cactus pear fruits and increased resistance of the cells to ex vivo induced oxidative hemolysis in humans. J Agric Food Chem. 2005;53:1266–70.
17. Liveri ML, Sciascia L, Lombardo R, Tesoriere L, Passante E, Livrea MA. Spectrophotometric evidence for the solubilization site of betalain pigments in membrane biomimetic systems. J Agric Food Chem. 2007;55:2836–40.
18. Turco Liveri ML, Sciascia L, Allegra M, Tesoriere L, Livrea MA. Partition of indicaxanthin in membrane biomimetic systems. A kinetic and modeling approach. J Agric Food Chem. 2009;57:10959–63.
19. Gentile C, Tesoriere L, Allegra M, Livrea MA, D'Alessio P. Antioxidant betalains from cactus pear (*Opuntia ficus-indica*) inhibit endothelial ICAM-1 expression. Ann N Y Acad Sci. 2004;1028:481–6.
20. Tesoriere L, Allegra M, Butera D, Livrea MA. Absorption, excretion, and distribution of dietary antioxidant betalains in LDLs: potential health effects of betalains in humans. Am J Clin Nutr. 2004;80:941–5.
21. Khoo KF, Tan HJ, Rosdinom R, Raymond AA, Norlinah MI, Shamsul A, Nafisah WY. Prevalence of depression in stroke patients with vascular dementia in universiti kebangsaan malaysia medical center. Med J Malaysia. 68:105–10.
22. Fraga CG. Plant polyphenols: how to translate their in vitro antioxidant actions to in vivo conditions. IUBMB Life. 2007;59:308–15.
23. Manach C, Williamson G, Morand C, Scalbert A, Remesy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr. 2005;81:230S–42S.
24. Di Rosa M, Willoughby DA. Screens for anti-inflammatory drugs. J Pharm Pharmacol. 1971;23:297–8.
25. Stintzing FC, Schieber A, Carle R. Identification of betalains from yellow beet (*Beta vulgaris* L.) and cactus pear [*Opuntia ficus-indica* (L.) Mill.] by high-performance liquid chromatography-electrospray ionization mass spectrometry. J Agric Food Chem. 2002;50:2302–7.
26. Ianaro A, Ialenti A, Maffia P, Pisano B, Di Rosa M. Role of cyclopentenone prostaglandins in rat carrageenin pleurisy. FEBS Lett. 2001;508:61–6.
27. Maffia P, Grassia G, Di Meglio P, Carnuccio R, Berrino L, Garside P, Ianaro A, Ialenti A. Neutralization of interleukin-18 inhibits neointimal formation in a rat model of vascular injury. Circulation. 2006;114:430–7.
28. Cuzzocrea S, Pisano B, Dugo L, Ianaro A, Maffia P, Patel NS, Di Paola R, Ialenti A, Genovese T, Chatterjee PK, et al. Rosiglitazone, a ligand of the peroxisome proliferator-activated receptor-gamma, reduces acute inflammation. Eur J Pharmacol. 2004;483:79–93.
29. Panza E, Tersigni M, Iorizzi M, Zollo F, De Marino S, Festa C, Napolitano M, Castello G, Ialenti A, Ianaro A. Lauroside B, a megastigmane glycoside from Laurus nobilis (bay laurel) leaves, induces apoptosis in human melanoma cell lines by inhibiting NF-kappaB activation. J Nat Prod. 74:228–33.
30. Thomsen LL, Ching LM, Zhuang L, Gavin JB, Baguley BC. Tumor-dependent increased plasma nitrate concentrations as an indication of the antitumor effect of flavone-8-acetic acid and analogues in mice. Cancer Res. 1991;51:77–81.
31. Kim H, Lee HS, Chang KT, Ko TH, Baek KJ, Kwon NS. Chloromethyl ketones block induction of nitric oxide synthase in murine macrophages by preventing activation of nuclear factor-kappa B. J Immunol. 1995;154:4741–8.
32. Gilroy DW, Colville-Nash PR, Willis D, Chivers J, Paul-Clark MJ, Willoughby DA. Inducible cyclooxygenase may have anti-inflammatory properties. Nat Med. 1999;5:698–701.
33. Liu SF, Malik AB. NF-kappa B activation as a pathological mechanism of septic shock and inflammation. Am J Physiol Lung Cell Mol Physiol. 2006;290:L622–45.
34. Visioli F, De La Lastra CA, Andres-Lacueva C, Aviram M, Calhau C, Cassano A, D'Archivio M, Faria A, Fave G, Fogliano V et al. Polyphenols and human health: a prospectus. Crit Rev Food Sci Nutr. 51:524–46.
35. Williamson G, Manach C. Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. Am J Clin Nutr. 2005;81:243S–55S.
36. Rotelli AE, Aguilar CF, Pelzer LE. Structural basis of the anti-inflammatory activity of quercetin: inhibition of the 5-hydroxytryptamine type 2 receptor. Eur Biophys J. 2009;38:865–71.
37. Ojewole JA. Antiinflammatory and analgesic effects of Psidium guajava Linn. (Myrtaceae) leaf aqueous extract in rats and mice. Methods Find Exp Clin Pharmacol. 2006;28:441–6.
38. Kanner J, Harel S, Granit R. Betalains–a new class of dietary cationized antioxidants. J Agric Food Chem. 2001;49:5178–85.
39. Frank T, Stintzing FC, Carle R, Bitsch I, Quaas D, Strass G, Bitsch R, Netzel M. Urinary pharmacokinetics of betalains following consumption of red beet juice in healthy humans. Pharmacol Res. 2005;52:290–7.
40. Tesoriere L, Gentile C, Angileri F, Attanzio A, Tutone M, Allegra M, Livrea MA. Trans-epithelial transport of the betalain pigments

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indicaxanthin and betanin across Caco-2 cell monolayers and influence of food matrix. Eur J Nutr. 52:1077–87.

41. Tesoriere L, Fazzari M, Angileri F, Gentile C, Livrea MA. In vitro digestion of betalainic foods. Stability and bioaccessibility of betaxanthins and betacyanins and antioxidative potential of food digesta. J Agric Food Chem. 2008;56:10487–92.
42. Kitano A, Matsumoto T, Oshitani N, Nakagawa M, Yasuda K, Watanabe Y, Tomobuchi M, Obayashi M, Tabata A, Fukushima R, et al. Distribution and anti-inflammatory effect of mesalazine on carrageenan-induced colitis in the rabbit. Clin Exp Pharmacol Physiol. 1996;23:305–9.
43. Bhattacharyya S, Borthakur A, Anbazhagan AN, Katyal S, Dudeja PK, Tobacman JK. Specific effects of BCL10 Serine mutations on phosphorylations in canonical and noncanonical pathways of NF-kappaB activation following carrageenan. Am J Physiol Gastrointest Liver Physiol. 301:G475–86.
44. D'Acquisto F, Ianaro A. From willow bark to peptides: the ever widening spectrum of NF-kappaB inhibitors. Curr Opin Pharmacol. 2006;6:387–92.
45. Ben-Neriah Y, Karin M. Inflammation meets cancer, with NF-kappaB as the matchmaker. Nat Immunol. 12:715–23.

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