Arzneim.-Forsch./Drug Res.

Effects of Phycocyanin Extract on Prostaglandin E_2 Levels in Mouse Ear Inflammation Test

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Summary

Recently it was demonstrated that phycocyanin, a biliprotein isolated from microalgae Spirulina, exerts anti-inflammatory activity in several animal models of inflammation. In this report, the effects of phycocyamin on prostaglandin E_2 (PGE_2) concentrations and phospholipase A_2 (PLA_2) activity were determined in arachidonic acid (AA) and 12-O-tetradecanoyl phorbol 13-acetate (TPA)-induced mouse ear oedema, respectively. Phycocyanin (50–200 mg/kg p.o.) inhibited in a dose-dependent manner PGE_2 levels in mouse ear treated with AA. Also, phycocyanin (100–400 mg/kg p.o.) moderately reduced PLA_2 activity in TPA-induced mouse ear inflammation test. In this model triamcinolone (10 mg/kg p.o.) used as reference drug exerted a remarkable inhibitory effect on PLA_2 activity. These results provide the first evidence that the anti-inflammatory effects of phycocyanin may result, at least partially, from inhibition of PGE_2 production and a moderate inhibition of PLA_2 activity.

Zusammenfassung

Wirkung von Phycocyanin-Extrakt auf die Prostaglandin E_2-Spiegel am Mausohrentzündungsmodell

Vor kurzem wurde gezeigt, daß Phykocyanin, ein aus Spirulina Microalgae isoliertes Gallenprotein, einen guten Effekt gegen Entzündung in mehreren Tiermodellen ausübt. In der vorliegenden Studie wird über die Phykocyanin-Wirkung auf Konzentrationen von Prostaglandin E_2 (PGE_2) und die Bestimmung der Aktivität von Phospholipase A_2 (PLA_2) beim Arachidonsäure (AA)- und 12-O-Tetradecanoyl-phorbol-13-acetat auf (TPA)-induziertem Mausohrödem berichtet. Phykocyanin (50–200 mg/kg p.o.) hemmt in dosisabhängiger Weise die PGE_2-Spiegel in mit AA behandelten Mausohren. Phycocyanin (100–400 mg/kg p.o.) verringert gemäßigt die PLA_2-Aktivität in TPA-induzierten Enzündungstests am Mausohr. Triamcinolon 810 mg/kg p.o.) das als Vergleichssubstanz eingesetzt wurde, zeigte einen bemerkenswerten Hemmeffekt auf die PLA_2-Aktivität. Diese Ergebnisse liefern den ersten Beweis dafür, daß die entzündungshemmenden Effekte von Phykocyanin zumindest teilweise auf der Hemmung der PGE_2-Erzeugung und der PLA_2-Aktivität beruhen.

Key words Anti-inflammatories · Phospholipase A_2 · Phycocyanin, anti-inflammatory effect, mouse · Prostaglandin E_2

Arzneim.-Forsch./Drug Res. 50 (II), 1106–1109 (2000)

1. Indroduction

Phycocyanin is a biliprotein found in blue green algae such as Spirulina and we demonstrated recently that it exerts scavenging action against reactive oxygen species (ROS) as well as anti-inflammatory activity in various in vitro and in vivo experimental models [1, 2, 3]. Furthermore, it was also reported that phycocyanin extract reduces leukotriene B_4 (LTB_4) levels in arachidonic acid (AA)-induced mouse ear inflammation test [4], which was ascribed to antioxidant properties of the pigment. Therefore, taking into consideration all these findings and to offer further insight in the participation of arachidonic acid metabolites in the mode of action of phycocyanin as anti inflammatory agent we decided to test phycocyanin comparatively in AA- and 12-O-tetradecanoyl phorbol 13-acetate (TPA)-induced mouse ear inflammation test and to determine the prostaglandin E_2 (PGE_2) concentrations and phospholipase A_2 (PLA_2) activities in ear tissue, respectively.

2. Materials and methods

2.1. Animals

Male OF_1 mice weighing 22–25 g were used in the experiments. The animals were purchased from the National Center for Laboratory Animal Production (Cenpalab, Havana, Cuba). The animals were housed in an environmentally (t = 25 °C) and air humidity (60 %) controlled room with a 12 h light-dark cycle, kept on a standard laboratory diet and drinking water ad libitum. The experiments were conducted in accordance with the ethical guidelines for investigations in laboratory animals and were approved by the Ethical Commitee for Animal Experimentation of the National Center for Scientific Research (CNIC).

2.2. Reagents

AA, TPA and PLA_2 from bovine pancreas (600 U/mg protein) were purchased from Sigma Chemical Co (St. Louis, MO, USA). PGE_2 Kit (enzymatic immunoassay system, EIA) was obtained from Amersham (England). Indometacin and triamcinolone diacetate were obtained from Merck (New Jersey, USA) and Lederle (PA, USA) respectively. Other reagents of analytical grade were purchased from normal commercial sources.

2.3. Preparation of phycocyanin extract

Phycocyanin was extracted from microalgae Spirulina (Arthrospira) máxima as described in a Cuban Patent [5]. The blue powder thus obtained showed a peak in the absorption spectrum of 620 nm, which is very close to the one reported for c-phycocyanin [6].

2.4. AA-induced mouse ear oedema

The method described by Opas et al. [7] was followed. Mice were fasted for 18 h with free access to water and divided into groups of 7 animals. Inflammation was induced by topical application of AA (0.5 mg/20 µl acetone) to the right ear of each mouse. Left ear (control) received the vehicle. Phycocyanin (50, 100 and 200 mg/kg in water) was administered by gavage 1 h before AA. The positive control group received indometacin, 1 mg/ear topically. Inflammation was followed for 1 h and thereafter animals were killed by cervical dislocation. A 6-mm section of ears was obtained and weighed. The swelling induced by AA was assessed as the increase in weight of ear punch of treated groups over untreated one and it was called the oedema index.

2.5. PGE_2 measurement

Ear tissue was weighed and immediately placed into 1 ml of ice-cold mixture of methanol / 0.1 mol/l sodium acetate, pH 4.2 (1 : 1) (v/v) and thereafter it was homogenized using an Ultra-turrax T-25 Polytron maintaining the tube into an ice bath. The homogenates were centrifuged for 10 min at 12,000 g at 4 °C. PGE_2 concentrations were determined reliably by an EIA kit which was used according to the instructions of the manufacturer. PGE_2 concentration was expressed in ng/ear.

2.6. TPA-induced mouse ear oedema

Oedema was induced in the right ear of mice by topical application of 4 µg/ear of TPA in acetone [8]. The left ear (control) received vehicle (acetone, 20 µl). Phycocyanin (100, 200 and 400 mg/kg) and triamcinolone (10 mg/kg), as reference group, were administered orally 1 h before TPA application. 6 h later, mice were killed by cervical dislocation and a 6 mm diameter disc from each ear was removed with a metal punch and weighed. The swelling induced by TPA was assessed as the increase in the weight of the right ear punch biopsy over that of the left ear and called the oedema index.

2.7. PLA_2 assay

Ear homogenates were prepared in 10 mmol/l Tris-HCl pH 7.4, 100 mmol/l KCl, 2 mmol/l $CaCl_2$ using an Ultra-turrax T-25 Polytron during 15 s. The homogenates were centrifuged at 2,500 g at 4 °C for 10 min. The supernatant was used to measure the PLA_2 activity according to Wittenauer et al. [9], using a fluorescent substrate, 1-acyl-2-[6(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-caproyl] phosphatidylcholine (C_6-NBD-PC). Reaction mixture contained C_6-NBD-PC (5×10^{-6} mol/l) in 1.0 mL of 10 mmol/l Tris-HCl, pH 7.4, 100 mmol/l KCl and 2 mmol/l $CaCl_2$. Fluorescence was measured in a Shimadzu RS-5000 spectrofluorimeter at an excitation and emission wavelength of 470 and 540 nm, respectively. An aliquot of sample containing 20–26 µg of protein was added to the test tube followed by incubation at 25 °C for 15 min and then fluorescence was measured. Activity of the enzyme was determined using bovine pancreatic PLA_2 of known activity (10–160 U) as standard.

2.8. Statistical analysis

Data are presented as means ± standard deviation. Mean differences between groups were compared by one-way analysis of variance (ANOVA) with the Duncan Multirange test. The level of statistical significance was taken as $p < 0.05$.

3. Results

3.1. Effects of phycocyanin on oedema and PGE_2 concentrations induced by AA in mouse ear

Phycocyanin (50–200 mg/kg p.o) significantly reduced in a dose-dependent manner both, oedema and PGE_2 concentrations in mouse ear treated with AA. Indometacin (1 mg/ear) administered topically and used as reference drug also significantly reduced oedema and PGE_2 concentrations in this test (Table 1).

Table 1: Effect of phycocyanin on oedema and PGE_2 concentration induced by arachidonic acid in mouse ear.

	Punch weight (mg)	Oedema index inhibition %	PGE_2/ear (ng)	Inhibition %
AA	14.0 ± 0.3	–	2.00 ± 0.04	–
Vehicle	7.0 ± 0.2	–	0.32 ± 0.01	–
Indometacin				
1 mg/ear	7.6 ± 0.2*	91.4	0.42 ± 0.005*	79.0
Phycocyanin				
50 mg/kg	11.2 ± 0.4*	40.0	1.25 ± 0.02*	37.5
100 mg/kg	10.4 ± 0.2*	51.4	1.04 ± 0.006*	48.0
200 mg/kg	8.7 ± 0.6*	75.7	0.57 ± 0.006*	71.5

The oedema and PGE_2 concentration were measured 1 h after treatment with AA. Oedema index indicates the increase in the weight of the punch biopsy of the right ear (treated with AA) over that of the left ear (control). Each value represents mean ± SD of 7 animals. * $p < 0.05$ compared with AA-treated group.

Table 2: Effect of phycocyanin on TPA-induced oedema and PLA_2 activity in the mouse ear.

	Punch weight (mg)	Oedema index inhibition %	PGE_2/ear (ng)	Inhibition %
TPA (4 µg/ear)	14 ± 0.57	–	3.8 ± 0.001	–
Vehicle	6 ± 0.33	–	0	–
Triamcinolone				
10 mg/kg	7 ± 0.16*	87.5	0.41 ± 0.009*	89.2
Phycocyanin				
100 mg/kg	13 ± 0.16*	12.5	3.4 ± 0.002*	10.5
200 mg/kg	12 ± 0.39*	25.0	2.9 ± 0.002*	23.6
400 mg/kg	11 ± 0.65*	37.5	2.3 ± 0.002*	39.4

The oedema and PLA_2 activity were measured 6 h after treatment with TPA. Oedema index indicates the increase in the weight of the punch biopsy of the rigth ear (treated with TPA) over that of the left ear (control). Each value represents mean ± SD of 7 animals. * $p < 0.05$ compared with TPA-treated group.

3.2. Effects of phycocyanin on TPA-induced oedema and PLA_2 activity in the mouse ear

As shown in Table 2 phycocyanin (100, 200 and 400 mg/kg p.o.) induced in a dose dependent fashion a moderate but significant inhibition of both oedema and PLA_2 activity in mouse ear treated with TPA. Triamcinolone (10 mg/kg p.o.) used as reference drug induced strong inhibition of oedema and PLA_2 activity in this test (Table 2).

4. Discussion

AA and TPA, a tumor promoting agent and proteinkinase C activator, are widely used to induce cutaneous inflammation in experimental animals. The initiation of inflammatory responses by metabolites of AA via cyclooxygenase (CO) and lipoxygenase (LO) pathways [7] and suppression of acute inflammatory responses by inhibitors of CO and LO [10, 11] established an important role for metabolites of AA in acute inflammation induced by AA and TPA, although Crummey et al. [12] also demonstrated that some free radical scavengers were able to markedly inhibit the edematous response to AA, which provide evidence in favor of involvement of ROS and radical mechanisms in inflammatory response induced by AA in mouse ear. We evaluated phycocyanin in this test and it significantly inhibited in a dose-dependent manner (50–200 mg/kg p.o.) both, oedema and PGE_2 concentrations in the mouse ear.

A possible cause of these effects might be inhibition of CO by phycocyanin. The role of hydroperoxides as the first step in arachidonic acid metabolism has been demonstrated. Lands [13] provided strong evidence that in inflammatory disorders the continual presence of lipid peroxide induces a free radical chain reaction mechanism, which sustains CO biosynthesis of more peroxides. Thus, hydroperoxides generated during arachidonic acid metabolism exert a positive feedback mechanism and thus stimulate CO activity.

This "peroxide tone" can be blocked by free radical scavengers and antioxidants, which act as reversible noncompetitive inhibitors of CO [14].

Previously we reported that phycocyanin is a ROS scavenger of hydroxyl and alkoxyl radicals and also inhibits microsomal lipid peroxidation induced by Fe^{2+}-ascorbate [1]. Regarding its reactivity towards peroxyl radicals, it has been shown that there is a significant bleaching of the chromophore fluorescence when the protein was exposed to the peroxyl radicals generated in the thermolysis of 2,2'-azobis (2-amidinopropane)hydrochloride (AAPH) [15]. Recently we obtained a body of data that allowed to evaluate the mechanism and kinetics of the bilin group destruction by peroxyl radicals. From this analysis we conclude that micromolar concentrations of phycocyanin are able to reduce the steady state concentration of the peroxyl radicals by one half, indicating the antioxidant activity of this compound. In line with the former findings, phycocyanin exerted protective effects against human erythocytes lysis induced by peroxyl radicals which was ascribed to its scavenging action against this radical in aqueous phase before they attack the erythrocyte membranes and by this way protect

membranes from the oxidative damage and subsequent lipid peroxidation in similar manner as trolox and ascorbic acid do [16].

Therefore, taking into consideration these results it is conceivable that reduction of PGE_2 levels found in AA-induced mouse ear oedema (Table 1) might be due to the modulatory effect of phycocyanin on hydroperoxide tone by scavenging peroxyl radicals and subsequent reduction of prostaglandin synthase activity. A similar mechanism was suggested by Alanko et al. [17] to explain the inhibition of PGE_2 formation by some phenols.

Taking into account that phycocyanin lowers both PGE_2 and LTB_4 levels in mouse ear inflamed by AA (Table 1, [4]) also an alternative mechanism, such as the inhibition of PLA_2, may be involved in these effects. Therefore, to elucidate it we tested phycocyanin in TPA-induced mouse ear inflammation. This test has been demonstrated as very suitable for the distinction of PLA_2 inhibitors from other AA metabolism enzyme inhibitors (CO and 5-LO) [18], when the pharmacological evaluation is performed in comparison with the AA test. Thus PLA_2 inhibitors induce a remarkable inhibition in the TPA test in contrast with their lack of effect in AA-induced ear oedema [17]. In our experiments, phycocyanin orally administered induced a remarkable reduction of oedema and PGE_2 concentration in the AA test (Table 1) whereas it moderately inhibited in a dose dependent manner oedema and PLA_2 activity in the TPA test (Table 2).

Taken together these results show that the inhibitory effect on PLA_2 activity as well as the antioxidant properties of phycocyanin contribute to its inhibitory mode of action on AA metabolism and hence its anti-inflammatory properties.

5. References

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