Effects of Phycocyanin Extract on Prostaglandin E₂ 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 phycocyanin on prostaglandin E₂ (PGE₂) concentrations and phospholipase A₂ (PLA₂) 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 PGE₂ levels in mouse ear treated with AA. Also, phycocyanin (100–400 mg/kg p.o.) moderately reduced PLA₂ 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 PLA₂ activity. These results provide the first evidence that the anti-inflammatory effects of phycocyanin may result at least partially, from inhibition of PGE₂ production and a moderate inhibition of PLA₂ activity.

Zusammenfassung

Wirkung von Phycocyanin-Extrakt auf die Prostaglandin E₂-Spiegel am Mausohrentzündungsmodell

Vor kurzem wurde gezeigt, daß Phycocyanin, ein aus Spirulina Microalge isoliertes Gallenprotein, einen guten Effekt gegen Entzündung in mehreren Tiermodellen ausübt. In der vorliegenden Studie wird über die Phycocyanin-Wirkung auf Konzentrationen von Prostaglandin E₂ (PGE₂) und die Bestimmung der Aktivität von Phospholipase A₂ (PLA₂) beim Arachidonsäure (AA)- und 12-O-Tetradecanoyl-phorbol-13-acetat (TPA)-induziertem Mausohrentzündungsmodell berichtet. Phycocyanin (50–200 mg/kg p.o.) hemmt in dosisabhängiger Weise die PGE₂-Spiegel in mit AA behandelten Mausöhrchen. Phycocyanin (100–400 mg/kg p.o.) verringert gemäßigt die PLA₂-Aktivität in TPA-induzierten Entzündungsmodellen am Mausohr. Triamcinolon (810 mg/kg p.o.) das als Vergleichsubstanz eingesetzt wurde, zeigte einen bemerkenswerten Hemmeffekt auf die PLA₂-Aktivität. Diese Ergebnisse liefern ein erstes Beweis dafür, daß die entzündungshemmenden Effekte von Phycocyanin zumindest teilweise auf der Hemmung der PGE₂-Erzeugung und der PLA₂-Aktivität beruhen.

Key Words  Anti-inflammatories · Phospholipase A₂ · Phycocyanin, anti-inflammatory effect · mouse · Prostaglandin E₂
1. Introduction

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 B4 (LTB4) 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 E2 (PGE2) concentrations and phospholipase A2 (PLA2) activities in ear tissue, respectively.

2. Materials and methods

2.1. Animals

Male OF1 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 controlled room with 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 Committee for Animal Experimentation of the National Center for Scientific Research (CNIC).

2.2. Reagents

AA, TPA and PLA2 from bovine pancreas (600 U/mg protein) were purchased from Sigma Chemical Co (St. Louis, MO, USA). PGE2 kit (enzymatic immunoassay system, EIA) was obtained from Amersham (England). Indomethacin 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) maxima as described in a Cuban Patent [5]. The blue powder thus obtained showed a peak in the absorption spectrum at 620 nm, which is very close to the one reported for c-phycocyanin [6].

2.4. AA-induced mouse ear edema

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 solution) 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 indomethacin, 1 mg/ml ear tipically. Inflammation was followed for 1 and 2 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 edema index.

2.5. PGE2 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 in an ice bath. The homogenates were centrifuged for 10 min at 12,000 g at 4°C. PGE2 concentrations were determined reliably by an EIA kit which was used according to the instructions of the manufacturer. PGE2 concentration was expressed in ng/ear.

2.6. TPA-induced mouse ear edema

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 edema index.

2.7. PLA2 assay

Ear homogenates were prepared in 10 mmol/l Tris-HCl pH 7.4, 100 mmol/l KCl, 2 mmol/l CaCl2 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 PLA2 activity according to Wittmauer et al. [9], using a fluorescent substrate, 1-acetyl-2-[6-nitro-2,1,3-benzoxadiazol-4-yl] amino]-caproyl phosphatidylcholine (Cy2,NBD-PC). Reaction mixture contained Cy2,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 CaCl2. 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 PLA2 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 Multiple Range test. The level of statistical significance was taken as p < 0.05.

3. Results

3.1. Effects of phycocyanin on edema and PGE2 concentrations induced by AA in mouse ear

Phycocyanin (50-200 mg/kg p.o.) significantly reduced in a dose-dependent manner both, edema and PGE2 concentrations in mouse ear treated with AA. Indomethacin (1 mg/ear) administered topically and used as reference drug also significantly reduced edema and PGE2 concentrations in this test (Table 1).
Table 1: Effect of phycocyanin on edema and PGE2 concentration induced by arachidonic acid in mouse ear.

<table>
<thead>
<tr>
<th></th>
<th>Punch weight (mg)</th>
<th>Oedema index inhibition %</th>
<th>PGE2/ear (ng)</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA Vehicle</td>
<td>14.0 ± 0.3</td>
<td>-</td>
<td>2.00 ± 0.04</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>7.0 ± 0.2</td>
<td>-</td>
<td>0.32 ± 0.01</td>
<td>-</td>
</tr>
<tr>
<td>1 mg/ear</td>
<td>7.6 ± 0.2*</td>
<td>91.4</td>
<td>0.42 ± 0.005*</td>
<td>79.0</td>
</tr>
<tr>
<td>Phycocyanin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>11.2 ± 0.4*</td>
<td>40.0</td>
<td>1.25 ± 0.02*</td>
<td>37.5</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>10.4 ± 0.2*</td>
<td>51.4</td>
<td>1.04 ± 0.005*</td>
<td>43.0</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>8.7 ± 0.6*</td>
<td>75.7</td>
<td>0.57 ± 0.006*</td>
<td>71.5</td>
</tr>
</tbody>
</table>

The oedema and PGE2 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 PLA2 activity in the mouse ear.

<table>
<thead>
<tr>
<th></th>
<th>Punch weight (mg)</th>
<th>Oedema index inhibition %</th>
<th>PGE2/ear (ng)</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPA (4 µg/ear)</td>
<td>11 ± 0.57</td>
<td>-</td>
<td>3.8 ± 0.001</td>
<td>-</td>
</tr>
<tr>
<td>Vehicle</td>
<td>6.2 ± 0.33</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Triamcinolone</td>
<td>7.2 ± 0.16*</td>
<td>87.5</td>
<td>0.41 ± 0.009*</td>
<td>89.2</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phycocyanin</td>
<td>13 ± 0.16*</td>
<td>12.5</td>
<td>3.4 ± 0.005*</td>
<td>10.5</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>12 ± 0.39*</td>
<td>25.0</td>
<td>2.9 ± 0.002*</td>
<td>22.6</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>11 ± 0.65*</td>
<td>37.5</td>
<td>2.3 ± 0.002*</td>
<td>39.4</td>
</tr>
</tbody>
</table>

The oedema and PLA2 activity were measured 6 h after treatment with TPA. Oedema Index indicates the increase in the weight of the punch biopsy of the right 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 PLA2 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 PLA2 activity in mouse ear treated with TPA. Triamcinolone (10 mg/kg p.o.) used as reference drug induced strong inhibition of oedema and PLA2 activity in this test (Table 2).

4. Discussion

AA and TPA, a tumor promoting agent and protein kinase 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 lipooxygenase (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 Crumley et al. [12] also demonstrated that some free radical scavengers were able to markedly inhibit the edematos 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 PGE2 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 Fe2+-ascorbate [1]. Regarding its reactivity towards peroxy radicals it has been shown that there is a significant bleaching of the chromophore fluorescence when the protein was exposed to the peroxy radicals generated in the thermolysis of 2,2'-azobis (2-aminopropane)hydrochloride (AAPH) [15].

Recently we obtained a body of data that allowed to evaluate the mechanism and kinetics of the bilin group destruction by peroxy radicals. From this analysis we conclude that micromolar concentrations of phycocyanin are able to reduce the steady state concentration of the peroxy radicals by one half, indicating the antioxidant activity of this compound. In line with the former findings, phycocyanin exerted protective effects against human erythrocytes lysis induced by peroxy 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 PGE2 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 synthesis activity. A similar mechanism was suggested by Alanko et al. [17] to explain the inhibition of PGE2 formation by some phenols.

Taking into account that phycocyanin lowers both PGE2 and LTD4 levels in mouse ear inflamed by AA (Table 1, [4]) also an alternative mechanism, such as the inhibition of PLA2, may be involved in these effects. Therefore, to elucidate if we tested phycocyanin in TPA-induced mouse ear inflammation. This test has been demonstrated as very suitable for the distinction of PLA2 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 PLA2 inhibitors induce a remarkable reduction 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 PGE2 concentration in the AA test (Table 1) whereas it moderately inhibited in a dose dependent manner oedema and PLA2 activity in the TPA test (Table 2).

Taken together these results show that the inhibitory effect on PLA2 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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