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Letter to the Editor

Phycocyanin Extract Reduces Leukotriene B_4 Levels in Arachidonic Acid-induced Mouse-ear Inflammation Test

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Recently we discovered that phycocyanin, a biliprotein found in blue green algae such as *Spirulina*, exerts scavenging action against reactive oxygen species as well as anti-inflammatory activity in various in-vitro and in-vivo experimental models (González et al 1999; Romay et al 1998a, b). Thus, we demonstrated that phycocyanin inhibited, in a dose-dependent manner (50–300 mg kg^{-1}, p.o), the inflammatory response induced in the following tests: glucose oxidase-induced inflammation in the mouse paw (Romay et al 1998a), arachidonic acid- and TPA-induced mouse ear oedema, carrageenan-induced rat paw oedema in intact and adrenalectomized animals, cotton-pellet granuloma in rat (Romay et al 1998b) and acetic acid-induced colitis in the rat. In this last test a substantial reduction in myeloperoxidase activity and neutrophil infiltration were found in rats with colitis treated with phycocyanin (González et al 1999). The anti-inflammatory effect of phycocyanin was greater in the arachidonic acid-induced ear oedema in mice than in the other tests quoted above. Taking into account these results, it is conceivable that inhibitory effects on arachidonic acid metabolism and particularly on the neutrophil chemotactic mediator, leukotriene B_4 (LTB_4), are involved in the inflammatory response. To our knowledge, no data are available about the effects of phycocyanin on this mediator of inflammation. Therefore, we decided to assess the effect of phycocyanin extract on LTB_4 concentration in arachidonic acid-induced mouse ear oedema, an experimental model very sensitive to inhibitors of the leukotrienes pathway of arachidonic acid metabolism (Tsuji et al 1998).

We used a crude phycocyanin extract obtained as previously described (Benitez et al 1997). Inflammation induced by topical application of arachidonic acid (0·5 mg) in mouse ear was evaluated as described elsewhere (Romay et al 1998b). Phycocyanin (50, 100 and 200 mg kg^{-1} in water; 10 mL kg^{-1}) was administered orally 1 h before arachidonic acid application. Positive control groups received topical application of catechol (0·5 mg/ear) or linoleic acid (1·04 mg/ear) together with arachidonic acid.

Inflammation was developed for 1 h and thereafter mice were killed by cervical dislocation. A 6-mm section of ear was obtained and weighed. Ear tissue was homogenized for LTB_4 measurement as described by Opas et al (1985). LTB_4 determinations were made by an enzyme immuno assay system using a kit purchased from Amersham.

In our experiments phycocyanin at doses of 50, 100 and 200 mg kg^{-1} inhibited significantly ($P < 0·05$), and dose-dependently, ear oedema and the LTB_4 levels induced by arachidonic acid. Catechol and linoleic acid exerted similar effects (Table 1).

These results may be ascribed to direct inhibitory effects of phycocyanin on 5-lipoxygenase, but this awaits further elucidation. However, we think it is more probable that antioxidative and reactive oxygen species scavenging properties of phycocyanin are strongly involved in the reduction of LTB_4 concentration in mouse ear. Crummey et al (1987) reported remarkable inhibitory activity of antioxidative and reactive oxygen species scavengers in the arachidonic acid ear test.

The effect of phycocyanin on LTB_4 concentration might be due to a direct reduction of both enzymatic and non-enzymatic lipid peroxidation (and hence arachidonic acid metabolism) as well as to a further reduction in the activity of lipoxygenases due to their requirement for (hydro)-peroxides in order to stimulate enzymatic function.

We have demonstrated that phycocyanin exerts antioxidative activity (Romay et al 1998a). Thus, it was able to scavenge hydroxyl ($OH^{\bullet}$) and alcoxyl

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Table 1. Effect of phycocyanin on oedema and LTB_4 concentration induced by arachidonic acid in mouse ear.

Treatment	Oedema index (mg)	Inhibition (%)	LTB_4/ear (pg)	Inhibition (%)
Arachidonic acid	6·1 ± 0·06	–	100·2 ± 0·51	–
Catechol	0·6 ± 0·06*	90·16	12·7 ± 0·30*	86·3
Linoleic acid	1·1 ± 0·05*	81·96	14·0 ± 0·40*	82·8
Phycocyanin 50 mg kg^{-1}	2·9 ± 0·05*	51·37	31·8 ± 0·20*	65·9
Phycocyanin 100 mg kg^{-1}	2·1 ± 0·08*	65·40	19·6 ± 0·21*	78·9
Phycocyanin 200 mg kg^{-1}	0·9 ± 0·05*	85·08	11·0 ± 0·22*	88·1
Untreated control	–	–	6·7 ± 0·16	–

The oedema and LTB_4 concentration were measured 1 h after treatment with arachidonic acid. Oedema index indicates the increase in the weight of the punch biopsy of the right ear (treated with arachidonic acid) over that of the left ear (control). Each value represents mean ± s.d. of 7 animals. *<0·05, compared with arachidonic acid-treated group.

($RO^{\cdot}$) radicals as well as to inhibit both liver microsomal lipid peroxidation and the chemiluminescence response of polymorphonuclear leukocytes activated by zymosan. Furthermore, phycocyanin exerted anti-inflammatory activity in glucose oxidase-induced oedema in mouse paw, an experimental model in which H_2O_2 and $OH^{\cdot}$ radical mediate the inflammatory response.

Taking all this into account our results provide evidence for the inhibitory effect of phycocyanin on LTB_4 biosynthesis, which seems to be due to antioxidative properties of the pigment.

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