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Protective effect of silymarin in antigen challenge- and histamine-induced bronchoconstriction in in vivo guinea-pigs

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Abstract

The effects of silymarin on bronchoconstriction induced by antigen challenge and on post-antigen challenge hyperresponsiveness to substance P were evaluated in sensitized guinea-pigs. Silymarin significantly decreased the bronchoconstriction due to antigen administration in the early phase of the response. In contrast, the dose–response curve for substance P recorded 1 h after antigen challenge was not modified by pretreatment with silymarin. The influence of the flavonoid on hyperresponsiveness to histamine in propranolol- and PAF (platelet-activating factor)-treated animals was also assessed. Silymarin did not affect hyperresponsiveness to histamine induced by either propranolol or PAF although it had inhibitory activity on the bronchial contractile response to the autacoid. These results suggest that silymarin has a protective effect in the early phase of allergic asthma, an effect, which may be related to a negative influence of the flavonoid on bronchial responsiveness to histamine.

Keywords: (*Silybum marianum*); Silymarin; Asthma; Hyperreactivity; (Guinea-pig)

1. Introduction

Silymarin, the active principle from the fruit of *Silybum marianum* is known as a therapeutic agent for the treatment of liver disorders, in view of its antioxidant and membrane-stabilizing effects. As free radical production has been demonstrated in several pathological states, the activity of silymarin and other biologically active flavonoids has been investigated in acute inflammation models in vivo (De la Puerta et al., 1996).

Silymarin has a protective effect against stress-induced gastric ulcer (Alarcon De La Lastra et al., 1992), in which leukotrienes are involved (Ogle and Cho, 1989). In contrast, the flavonoid mixture is ineffective against ethanol-induced ulcer, a condition that is independent of the lipooxygenase pathway (Boughton-Smith and Whittle, 1988). An inhibitory action on lipooxygenase and on prostaglandin synthetase has been demonstrated in in vitro assays (Fiebrich and Koch, 1979a,b), in the absence of an inhibition of phospholipase A_2 (De la Puerta et al., 1996); silibinin has been shown to inhibit leukotriene formation also in human cells (Dehmlow et al., 1996).

Silymarin thus seems to possess antiinflammatory properties by acting through different mechanisms such as its antioxidant action, membrane-stabilizing effect and inhibition of the production or release of inflammatory mediators such as arachidonic acid metabolites. This complex activity seems to justify the popular use of *S. marianum* extracts for the treatment of allergic states and asthma, but this has not been validated by experimental results.

Pulmonary inflammation is considered to be an important component in the pathogenesis of asthma, and the stabilization of mastocyte membranes represents a known target of antiasthmatic drugs.

A role for lipooxygenase has been demonstrated in allergic asthma: in sensitized guinea-pigs, an animal model of allergic asthma, leukotrienes are implicated in the bronchoconstriction induced by antigen challenge (Malo et al., 1994) and in the aspecific bronchial hyperreactivity in both intrinsic and extrinsic asthma (Seeds et al., 1995), as well as in propranolol-(Ney, 1983) or platelet-activating factor (PAF)-induced (Seeds et al., 1995) hyperreactivity.

The aim of the present work was thus to study the effects of silymarin on asthma. In particular, the activity of sily-

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marin was examined against bronchial anaphylaxis and against post-anaphylactic, propranolol- or PAF-induced hyperreactivity in guinea-pigs.

2. Materials and methods

2.1. General procedure

Male albino guinea-pigs, weighing 350–500 g, were used in agreement with Italian Government regulations (D.L. 27/01/1992 no. 116) concerning the care and use of laboratory animals; the protocol was approved by the institutional ethics committee and complies with the European Community guidelines for the use of experimental animals.

The animals were anaesthetized with sodium pentobarbital (50 mg/kg b.w., i.p.). When a complete loss of reflexes was obtained, the trachea was cannulated and connected to a ventilation pump (Basile mod. 7025) and a pressure transducer (Bentley-Trantec mod.800). Mechanical responses were registered with a microdynamometer (Basile mod. 7050). The ventilation parameters were the following: 50 strokes/min, 1 ml air/100 g b.w. for each air stroke.

The jugular vein was also cannulated to allow intravenous administration of drugs. After the surgical procedure had been completed, pancuronium bromide 2 mg/kg b.w. was administered i.v. to abolish spontaneous breathing. At the end of the experiments, the animals were killed with a lethal dose of barbiturates i.v.

2.2. Anaphylaxis-induced hyperreactivity to substance P.

Hyperreactivity to acetylcholine and substance P after antigen challenge has previously been described in anaesthetized guinea-pigs (Nieri et al., 1992), in agreement with data reported by Daffonchio et al. (1988). The same experimental model was used in the present study, and substance P was chosen as the bronchoconstrictor.

Briefly, ovalbumin-sensitized guinea-pigs were used. The sensitization procedure was performed by the administration of two doses of ovalbumin (100 mg/kg b.w.), one subcutaneously and one intraperitoneally. 15–20 days after this treatment, the experiments were carried out in accordance with the following protocol: after the surgical procedure, treatment with the curarizing drug and a stabilization period of 15 min, guinea-pigs were exposed to ovalbumin aerosol (ovalbumin solution: 100 mg/ml; time: 30 s) or to ovalbumin solvent by means of an ultrasonic nebulizer (Aerosol Therapy Ultrasound System-Artsana) connected to the inspiration pipe of the ventilator pump. 1 h after the anaphylactic shock, a dose–response curve for substance P (5–80 μg/kg b.w.) was recorded. Some guinea-pigs were pretreated with silymarin (100 mg/kg b.w., i.p.), while others were administered the vehicle by the same route, 15 min before the beginning of the surgical procedure, corresponding to 1 h before the challenge with ovalbumin.

2.3. Propranolol-induced hyperreactivity to histamine

In nonsensitized guinea-pigs, two dose–response curves for histamine (5–80 μg/kg b.w., i.v.) were recorded: the first was the control response and the second was used to test the efficacy of drugs on this response.

The interval between the two dose–response curves for histamine was 30 min. 10 min before the second dose–response curve was recorded, the animals were treated with propranolol 1 mg/kg b.w., i.v. or with the vehicle of the β-adrenoceptor antagonist. The efficacy of silymarin against the propranolol-induced hyperreactivity to histamine was tested by administering the flavonoid before the first dose–response curve was recorded; controls were administered the flavonoid vehicle.

2.4. PAF-induced hyperreactivity to histamine

The experimental protocol was the same as that described for propranolol-induced hyperreactivity, the only difference being the supply of PAF (50 ng/kg b.w., i.v.) 10 min before the second dose–response curve for histamine instead of propranolol. Control preparations were treated with PAF and silymarin vehicles.

2.5. Analysis of data

Functional responses are given as pulmonary insufflation pressure values and dose–response curves were plotted by means of the computer program Prism 3.0 (GraphPad Software).

Data are expressed as arithmetic means ± S.E.M.; the statistical significance ($P \leq 0.05$) of the differences observed between treatments was evaluated by Student's *t*-test for paired and unpaired data depending on the experimental protocols (comparison between curves obtained in the same animal or in different animals).

2.6. Drugs and solutions

The following substances were used: histamine bisulphate, silymarin, DL-propranolol hydrochloride, substance P, PAF (platelet-activating factor), ovalbumin (Sigma, Italy), sodium pentobarbital (C. Sessa, Italy), pancuronium bromide (Pavulon, Organon Teknika, Italy). Silymarin solution was prepared by dissolving the drug in Tween 80 (0.8% in saline); PAF was dissolved in a solution of 0.25% bovine serum albumin in saline. All the other drugs were dissolved in saline.

3. Results

In sensitized guinea-pigs, the aerosol antigen challenge induced an immediate bronchospasm and a subsequent increase in pulmonary inflation pressure to 33.92 ± 4.90

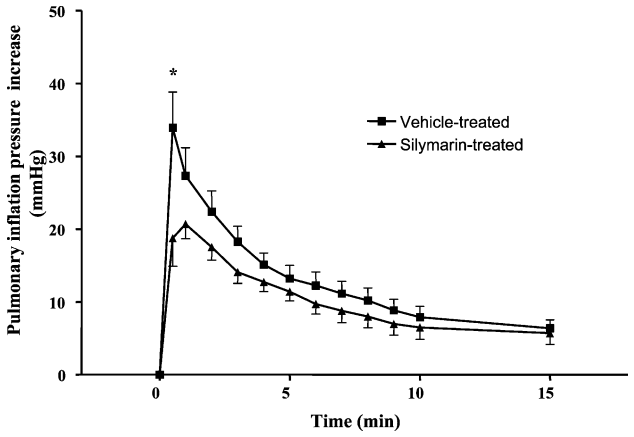

Fig. 1. Time course of bronchoconstriction following ovalbumin (OA) challenge (30 s aerosol with 100 mg/ml OA solution) in sensitized guinea-pigs. Comparison between vehicle (■) and silymarin-pretreated (▲) animals in the response to the antigen, measured as the increase in pulmonary inflation pressure. Each point represents the mean value ± S.E.M. (vertical bars) of eight experiments.

mm Hg, followed by a progressive but slow decrease in pulmonary inflation pressure to the basal value. Fig. 1 shows the pulmonary inflation pressure levels related to time after antigen administration. When the curves for silymarin-treated guinea-pigs and for control animals were compared, a significant decrease in the maximal increase in pulmonary inflation pressure (20.70 ± 2.01 mm Hg) was evident, together with a slight delay in the appearance of maximal bronchoconstriction (Fig. 1). No significant difference was observed between the subsequent time-related bronchoconstriction profiles.

Fig. 2 shows the dose–response curve for substance P 1 h after anaphylactic shock in vehicle-treated and in silymarin-treated animals. The two curves were found not to be statistically different, demonstrating that silymarin failed to prevent the hyperreactivity to substance P after antigen challenge.

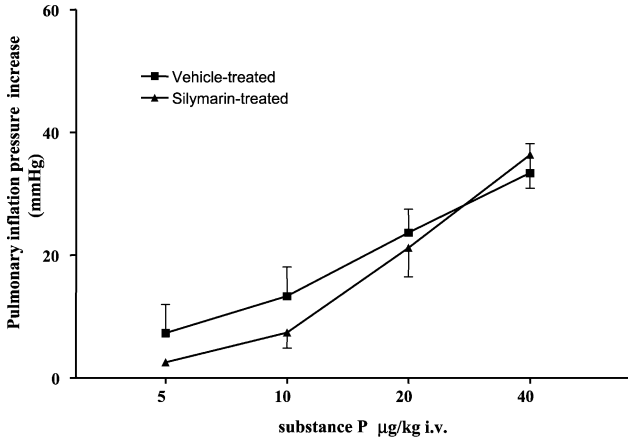

Fig. 2. Effect of silymarin on the bronchoconstrictive response for substance P. Dose–response curves to substance P in sensitized guinea-pigs challenged with ovalbumin 1 h before administration of the peptide: (▲) vehicle; (■) silymarin (100 mg/kg b.w. i.p.)–treated animals. Each point represents the mean value ± S.E.M. (vertical bars) of 5–8 experiments.

In propranolol- or PAF-induced hyperreactivity models, guinea-pigs were administered histamine to evaluate an increase in responsiveness to this agonist; thus, two dose–response curves for histamine were recorded at an interval of 30 min. In control animals treated with the solvent, the second dose–response curve for histamine was significantly shifted to the right with respect to the first one, with a significantly reduced increase in pulmonary inflation pressure at all the tested doses of histamine except the smallest one (5 μg/kg b.w., i.v.). Propranolol administration (1 mg/kg b.w., i.v.) before the second dose–response curve for histamine was recorded caused a strong increase in the response to the autacoid (Fig. 3A): the response for histamine 80 μg/kg was increased from 79.6 ± 0.2% in control animals to 221.5 ± 50.9% in propranolol-pretreated guinea-pigs. The administration of PAF before the second dose–

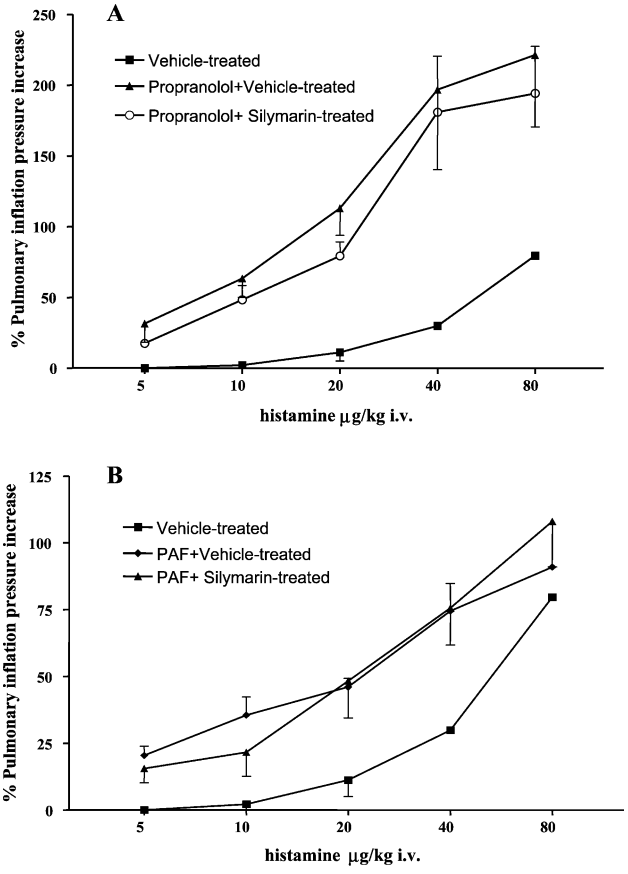

Fig. 3. Effect of silymarin on propranolol-induced (A) and PAF-induced (B) hyperreactivity to histamine in vivo in guinea-pigs. (A) Dose–response curves for histamine in the presence of silymarin vehicle (■), propranolol (1 mg/kg b.w., i.v.) + silymarin vehicle (▲) and propranolol (1 mg/kg b.w., i.v.) + silymarin (100 mg/kg b.w., i.p.) (○). (B) Dose–response curves for histamine in the presence of PAF and silymarin vehicles (■), PAF (50 ng/kg b.w., i.v.) + silymarin vehicle (♦) and PAF (50 ng/kg b.w., i.v.) + silymarin (100 mg/kg b.w., i.p.) (▲). Each point represents the mean value ± S.E.M. (vertical bars) of 5–8 experiments.

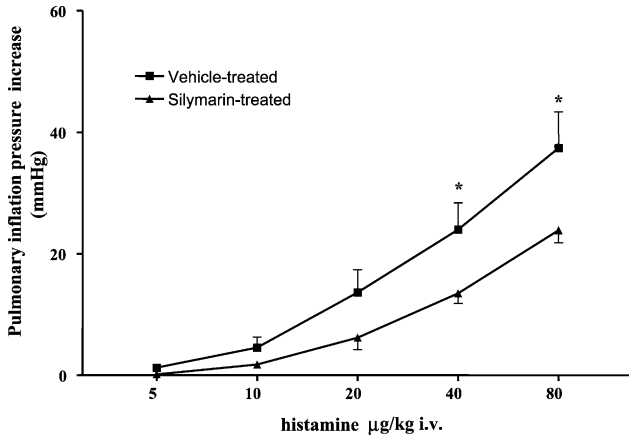

Fig. 4. Dose–response curve for histamine in silymarin (100 mg/kg b.w., i.p.)-pretreated guinea-pigs (▲), compared with control animals after vehicle administration (■). The flavonoid was administered 15 min before the amine. Each point represents the mean value ± S.E.M. (vertical bars) of 6–9 experiments. * $P \leq 0.05$.

response curve for histamine potentiated responsiveness to the autacoid although the response was smaller in comparison with that caused by propranolol administration: the maximal response to histamine was found to be 90.9 ± 0.6% (Fig. 3B).

Silymarin pretreatment (100 mg/kg b.w., i.p., 1 h before histamine administration) did not significantly influence either propranolol- or PAF-induced hyperreactivity, since the respective dose–response curves did not show any significant difference between untreated hyperreactive animals and hyperreactive animals treated with the flavonoid (Fig. 3A,B).

Both propranolol and PAF elicited a slight increase in pulmonary inflation pressure soon after their administration and before histamine bronchoprovocation. Thus, the influence of silymarin was examined on these responses, but no significant difference was revealed between control and silymarin-pretreated guinea-pigs.

When the responsiveness to histamine was studied, the first curve for the autacoid in controls was compared with that obtained in silymarin-pretreated guinea-pigs. The relative dose–response curves are shown in Fig. 4; a significant decrease in the responsiveness to histamine was seen in flavonoid-pretreated animals, albeit at the highest doses of histamine (40 and 80 μg/kg b.w., i.v.).

4. Discussion

Silymarin showed a moderately protective effect against the bronchospasm induced by aerosol antigen challenge in sensitized guinea-pigs. This action can be explained by the various biological effects of silymarin, i.e. to say, its membrane-stabilizing effect (Miadonna et al., 1987), its antinflammatory activity demonstrated in in vivo preparations (De la Puerta et al., 1996) and its inhibition of the arachidonic acid pathway (Fiebrich and Koch, 1979a,b). The decrease in the bronchospasm induced by antigen challenge was evident in the first phase of the response, indicating that the flavonoid acts mainly on the early inflammatory reaction that involves histamine release. A similar effect was demonstrated by quercetin, a flavonoid compound (Dorsch et al., 1992). Both quercetin (Middleton and Drzewiecki, 1984) and silibinin (Miadonna et al., 1987) were shown to inhibit antigen-stimulated histamine release from human basophil leukocytes. In acute inflammation models, silymarin demonstrated an antioedema effect related to an inhibitory action on leukocyte migration (De la Puerta et al., 1996). This mechanism cannot explain the efficacy of silymarin in reducing the antigen-induced bronchospasm, which is an immediate reaction in which the participation of histamine and substance P have been demonstrated (Hessel et al., 1995; Nieber et al., 1991). Substance P, a neurokinin released at the bronchial level from capsaicin-sensitive neurons, is known to have a significant role in asthma (Barnes, 1987) and a substance-P-induced hyperreactivity 1 h after anaphylactic shock has been demonstrated in sensitized anaesthetized guinea-pigs (Nieri et al., 1992). Silymarin did not affect the hyperreactivity to substance P, indicating that its ability to reduce bronchospasm in anaphylactic shock was unrelated to an influence on airway responsiveness to the neurokinin. In spite of this, an effect of the flavonoid on mechanisms or substances that are implicated in the hyperreactive phenomenon was not excluded and, consequently, silymarin efficacy was tested in different models of hyperresponsiveness such as propanolol and PAF-induced hyperreactivity in in vivo guinea-pigs, in which a role for leukotrienes has been demonstrated in the hyperresponsiveness to both agents (Seeds et al., 1995; Ney, 1983).

Silymarin did not show any protective effect against the hyperresponsiveness to histamine in either propanolol- or PAF-treated animals. This evidence seems to exclude the possibility that silymarin acts on leukotriene production in airways in vivo, in spite of its previously demonstrated inhibitory action on lipooxygenase in vitro (Fiebrich and Koch, 1979a; Dehmlow et al., 1996).

Our results demonstrate that silymarin does not influence the hyperreactive phenomenon although it decreases histamine-induced bronchoconstriction. A direct effect of the flavonoid on the bronchoconstriction induced by histamine has been suggested to explain the hyporesponsiveness to the autacoid, following silymarin administration in vivo. Some flavonoids have been shown to inhibit agonist-induced contraction of ileal smooth muscle from normal and ovalbumin sensitized guinea-pigs (Macander, 1986; Fanning et al., 1983). In spite of this, some experiments carried out on isolated bronchial preparations did not reveal any significant difference between control and silymarin-treated tissues (data not shown).

In conclusion, silymarin pretreatment reduced the bronchospasm induced by antigen-challenge in sensitized guinea-pigs, and this effect suggests that the flavonoid can be used

as protective agent in the management of asthmatic disorders. This protective effect seems to be due to an indirect mechanism that reduces airway responsiveness to histamine, and consequently the immediate anaphylactic response.

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