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Inhibition of antigen-induced lung anaphylaxis in the guinea-pig by BN 52021 a new specific paf-acether receptor antagonist isolated from Ginkgo biloba

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Abstract

Paf-acether appears to be a potent mediator released in response to allergen exposure in sensitized animals and it could contribute to clinical manifestations of allergic asthma. In order to ascertain this assumption the inhibition of antigen-induced lung anaphylaxis in guinea-pig by BN 52021, a new highly specific paf-acether antagonist, was studied. Ovalbumin injected into passively sensitized guinea-pig induced a large bronchoconstriction which was accompanied by thrombocytopenia and leukopenia. Treatment of animals with BN 52021 i.v., five minutes before challenge, strongly (at the doses of 1 and 2 mg/kg) or totally (at 0.1 mg/kg) inhibited the bronchoconstriction and partially reduced the thrombocytopenia and leukopenia the thrombocytopenia occurring after challenge. These results confirm that paf-acether and platelets might play a key role in asthma. BN 52021 and other paf-acether antagonist could provide a new group of potent prophylactic anti-asthma drugs.

Introduction

Paf-acether (Platelet-activating factor, 1-0-alkyl-2-0-acetyl-sn-3-phosphorylcholine, PAF) is one of the most potent inducers of platelet aggregation known [1]. It was first characterized as a product of rabbit basophils following IgE-dependent activation [2] but production of paf-acether is now known to accompany immune activation of other cell types such as neutrophils, vascular endothelial cells or IgE-sensitized macrophages exposed to specific antigens. These cells are thus potential sources for the formation of paf-acether in pathophysiological conditions. Since paf-acether induces bronchoconstriction when injected i.v. in to the guinea-pig [3] and since it is released during immediate hypersensitivity, lung anaphylaxis and shock [4], it apparently plays a major role in the triggering and/or in the exacerbation of asthma symptoms.

Unfortunately, direct evidence for a role of paf-acether in lung pathophysiology was not provided until now, mainly due to the lack of specific antagonists. These drugs are at present becoming available and in this paper we describe the properties of BN 52021, a new highly specific paf-acether antagonist which is a chemically defined molecule (Fig. 1) extracted from Ginkgo biloba leaves or from the standardized Ginkgo biloba extract GBE 761 [5].

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	R_1	R_2	R_3
BN 52020	OH	H	H
BN 52021	OH	OH	H
BN 52022	OH	OH	OH

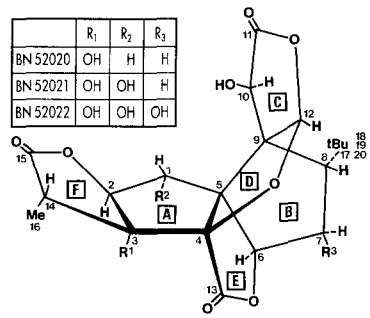

Figure 1
Structure of BN 52021.

This compound appears as a potent and specific inhibitor of the binding of [^{3}H] paf-acether to its receptor [5] and of human and rabbit platelet aggregation *in vitro* and *ex vivo* [5]. BN 52021 also suppresses paf-acether-induced bronchoconstriction in guinea-pig [6]. In this paper, we studied the antagonism by BN 52021 of antigen-induced lung anaphylaxis in the guinea pig.

Materials and methods

Male Hartley guinea-pigs (400–500 g) were sensitized by i.v. injection of 1 ml/kg of diluted heterological rabbit anti-ovalbumin immune serum (Cappel, USA) in order to obtain 75% bronchoconstriction upon challenge. In this condition, bronchoconstriction might be mediated by immunoglobulin G. After 24 hours, the animals were anaesthetized with urethane (2 g/kg, i.p.), tracheotomized and placed under assisted respiration (80 strokes/min – 1 ml air/100 g per stroke).

A pneumothorax was carried out to avoid any spontaneous respiration. The right jugular vein was catheterized for the injections. In accordance with Konzett and Rössler's method, the initial resistance to the insufflation was maintained at 10 cm H_2O, the excess air volume being measured using a bronchospasm transducer (Ugo Basile) connected to a GEMINI recorder.

Platelets and leukocytes were counted with a Coulter counter (ZM type). For this purpose, arterial blood was

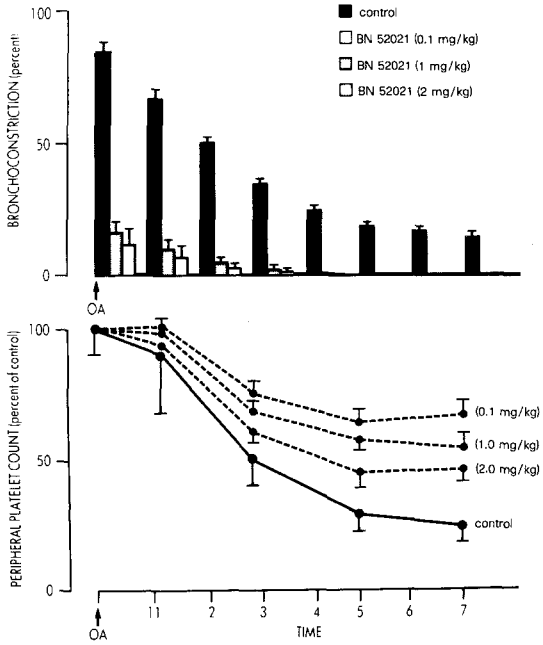

Figure 2
Effect of BN 52021 on bronchoconstriction in passive immune anaphylactic shock.
Drug was given preventively 5 min before challenge by i.v. route. Sensitization was realized by i.v. injection of diluted heterological rabbit antiovalbumin immune serum 24 hours before challenge with ovalbumin.

collected in heparinized tubes beforehand and four times after the i.v. injection of ovalbumin (1.5, 10 and 15 min).

BN 52021 (0.1, 1 and 2 mg/kg, i.v.) and FPL 55712 (1 and 2 mg/kg, i.v.) were administered 5 min before the injection of ovalbumin (1 mg/kg, i.v.). The bronchoconstriction was expressed as a percentage of the effect obtained by clamping the trachea at the end of the experiment. The results obtained were compared to the controls injected with isotonic sodium chloride.

In all experiments, the inhibitory effect of the products was calculated as follows:

Inhibition (%): $(1-D/c) \times 100$ where:

c = average of the bronchoconstriction of the control animals.

D = average of the bronchoconstriction of the treated animals.

Results and discussion

The injection of ovalbumin in passively sensitized guinea-pig induced a large bronchoconstriction which was accompanied by significant thrombocytopenia and leukopenia. These two phenomena were both delayed compared with the time of maximum bronchospasm amplitude. In a limited number of animals, it appeared that a transient increase in the number of platelets preceeded thrombocytopenia.

In control animals, ovalbumin (1 mg/kg) induced a submaximum bronchoconstriction (75.7 ± 4.8%) compared to 100% obtained by clamping. This dose was chosen to study the effect of BN 52021. Injected by the i.v. route 5 min before the challenge, BN 52021 inhibited the bronchospasm by 78.2% and 84.7% for 2 and 1 mg/kg respectively.

At 0.1 mg/kg, BN 52021 completely abolished the immunological response (Fig. 2). Under the same experimental conditions, comparatively, FPL 55712 (2 mg/kg, i.v., 5 min before challenge), a leukotriene receptor antagonist inhibited the bronchoconstriction by only 48% and was totally inactive at lower doses ($\leqslant$ 1 mg/kg). The inhibition of the bronchospasm by BN 52021 was accompanied by a partial recovery of the leukocyte and platelet counts (data not shown).

VARGAFTIG *et al.* [3] have shown that injection of paf-acether induced a significant bronchoconstriction associated with a transient thrombocytopenia which was maximum in the first minutes following the challenge. In our experiments, the antigen-induced bronchoconstriction also appeared rapidly but the thrombocytopenia was delayed in comparison with that following paf-acether challenge. In all cases maximum bronchoconstriction preceeded thrombocytopenia. Furthermore thrombocytopenia lasted longer than bronchospasm. This suggests that the bronchoconstriction mechanisms may be different from those responsible for the peripheral drop of platelet counts due to sequestration of platelets in the pulmonary vasculature. The fact that BN 52021 strongly inhibited the bronchoconstriction occurring in anaphylactic shock is strong evidence that antigen challenge induced the release of paf-acether or paf-acether like products in the lung. Our results also corroborate MORLEY and colleagues' hypothesis [7] that platelets could play a key role in asthma, since BN 52021 also partially inhibited the important thrombocytopenia occurring during the anaphylactic response.

BN 52021 and other paf-acether antagonists could provide a new group of potent prophylactic anti-asthma drugs.

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