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EFFECT OF CYCLOSPORIN A AND THE PLATELET-ACTIVATING FACTOR (PAF) ANTAGONIST, BN 52021, ON PAF- AND ANTIGEN- INDUCED BRONCHOCONSTRICTION IN THE GUINEA-PIG

Caroline TOUVAY, Béatrice VILAIN, Valérie LEJEUNE, Jean Michel MENCIA-HUERTA and Pierre BRAQUET

Department of Immunology, Institut Henri Beaufour, 1 avenue des Tropiques, 91952 LES ULIS CEDEX, France

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The effects of the PAF antagonist, BN 52021, and cyclosporin A (CsA), either alone or in combination, on PAF- and antigen- induced bronchoconstriction were investigated in control and passively sensitized guinea-pigs, respectively. Although single administration of CsA alone has no effect on the PAF-induced bronchoconstriction, a marked inhibition of this phenomenon is observed when the drug is given along with an inactive dose of BN 52021. This effect of the association of the two drugs on the bronchoconstriction is also related to an action on the PAF-induced alterations in the number of leukocytes and platelets. In addition, administration of CsA for 48 hrs, which alone does not influence PAF-induced bronchoconstriction, markedly increases the inhibition evoked by BN 52021. Although bolus administration of CsA has no effect on the antigen -induced bronchoconstriction, a marked inhibition of this phenomenon is observed when the drug is given for 2 days. This inhibition by CsA is not further enhanced when the animals are also treated with BN 52021. These results strengthen the hypothesis that PAF and the immune system are involved in the regulation of bronchopulmonary reactions.
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The role of immunocompetent cells (T and B lymphocytes, monocytes) in the regulation of anaphylactic reactions is now the center of active investigations. Indeed, following antigen challenge, marked alterations in lymphocyte populations are observed, both in the broncho-alveolar lavage fluids of the guinea-pig and in the human skin (1-3). However, the precise contribution of these cell types in the control of the bronchopulmonary response is not yet determined. Cyclosporin A (CsA), a product of the fungus Tolypocadium inflatum gams, is a potent immunosuppressive drug (4-6) used in transplantation and for the treatment of several immune-mediated diseases (7, 8). In addition, CsA has been shown to be a potent inhibitor of histamine release from human basophil leukocytes and isolated rat mast cells (9), suggesting that the drug possesses a broad spectrum of activities. This latter result also indicate that this drug might interfere with the development of antigen-induced bronchoconstriction.

Recently, a role for PAF in the regulation of immune processes was reported (10, 11, reviewed in 12). Interestingly, treatment of rats with the

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specific PAF antagonist, BN 52021, in association with CsA improves cardiac graft survival compared to the administration of the immunosuppressive drug alone (13). More recently, Pignol et al. (14) have demonstrated that oral administration of BN 52021 to rats treated with CsA further enhances the effect of low doses of the immunosuppressive drug on lymphocytes proliferation and interleukin 2 production. In addition, works by Pirotzky et al. (15, 16) have shown that treatment of spontaneously hypertensive rats with BN 52063, a mixture of PAF antagonists including BN 52021, markedly reduces the renal alterations induced by CsA. Taken together, these data suggest a relationship between PAF and CsA actions. The aim of the present study was to investigate the effect of CsA and BN 52021, either alone or in combination, on PAF- and antigen- induced bronchoconstriction in the guinea-pigs in an attempt to determine a possible interaction of these two drugs at the bronchopulmonary level.

MATERIALS AND METHODS

Passive sensitization of the guinea-pigs.

Male Hartley guinea-pigs (400-450 g, Charles River, St Aubin les Elbeuf, France) were passively sensitized 18 to 24 h before challenge by i.v. injection of 1 ml/kg diluted (1:4 v/v) IgG-rich rabbit anti- ovalbumin antiserum (Cappel, Cooper Biomedical, Malvern, PA, USA).

Treatments of the animals with CsA and BN 52021.

- Acute treatment: Two hours before the experiments, CsA (Sandoz, Basel, Switzerland; 25 mg/kg) and BN 52021 (17)(IHB Research Laboratories, Le Plessis-Robinson, France; 15 mg/kg), either alone or in combination, were prepared in groundnut oil and given orally (1 ml/kg). Control guinea-pigs received 1 ml/kg of the vehicle alone, groundnut oil.

- Chronic treatment: The drugs in groundnut oil were given orally twice a day from 48 hours before the beginning of the experiments. A total of five administrations was given with the following doses : CsA (12,5 mg/kg) and BN 52021 (15 mg/kg), either alone or in combination. Control guinea-pigs received 1 ml/kg of the vehicle alone.

Preparation of the animals and determination of PAF- and antigen- induced bronchoconstriction, thrombocytopenia and leukopenia.

Male Hartley guinea-pigs (400-450 g) were anesthetized with ethylcarbamate (Prolabo, Paris, France; 1.2 g/kg, i.p.). The right jugular vein was catheterized for injections and the animals were tracheotomized and ventilated with a respiratory pump (Ugo Basile, Varese, Italy) (70-80 breathes/min, 1 ml air/100 g body weight/breath). A pneumothorax was performed to suppress spontaneous breathing. The initial inflation pressure was set constant at 10 cm water in accordance with the Konzett and Rössler's method (18) and the air excess was measured with a bronchospasm transducer (Ugo Basile) connected to a recorder (Gemini, Ugo Basile). The right carotid artery was cannulated for blood sampling. Blood (200 µl) was collected for the determination of leukocyte and platelet counts with a Coulter Counter ZBI, before and 1 min, 5 min and 10 min after the administration of PAF (1-0-hexadecyl-2- 0-acetyl- sn-glycero-3- phosphocholine, Novabiochem, Cléry en Vexin, France) or ovalbumin (OA, Fluka, Buchs, Switzerland). Bronchoconstriction was induced by i.v. injection of either 60 ng/kg PAF, or 0.75 mg OA/kg, i.v. when passively sensitized guinea-pigs were used. PAF

was prepared and stored at - 20°C as a stock solution in 0.9 % NaCl (saline) containing 0.25 % bovine serum albumin (BSA, fraction V, Sigma St. Louis, MO, USA). Dilutions were made daily in a 0.15 % BSA-containing saline solution.

The bronchoconstriction was expressed as a percentage of the value obtained by clamping the trachea at the end of the experiment (maximal bronchoconstrictor response). In all experiments, the inhibitory effect of the products was calculated as follows : inhibition (%) = (1 - B/A) x 100, where A and B are the average bronchoconstrictions in the groups of untreated and treated animals. Data are presented as the means ± standard errors of the mean (SEM). The statistical difference between mean values was assessed by variance analysis.

RESULTS

Effect of treatment with CsA and BN 52021, given alone or in combination, on PAF-induced bronchoconstriction, leukopenia and thrombocytopenia.

Injection of PAF to guinea-pigs induced a large bronchoconstriction that was unaffected by pretreatment of the animals with CsA or BN 52021 alone, given 2 hours before challenge with the autacoid (Fig. 1 A). In contrast, when the animals were treated with a combination of these two drugs, a marked and significant inhibition of 67.9 % was observed (Fig. 1 A). Combined treatment with both compounds also inhibited the PAF-induced thrombocytopenia (average inhibitions of 44 %, $p < 0.001$ at t = 1 min; 56 %, $p < 0.01$ at t = 5 min and 68 %, $p < 0.001$ at t = 10 min; n = 16), whereas these drugs were ineffective when administered separately. As well, the transient leukopenia following PAF injection was unaffected by treatment with BN 52021 or CsA alone but was strongly reduced by an average of 75 % by 5 and 10 min on treatment of the guinea-pigs with both compounds.

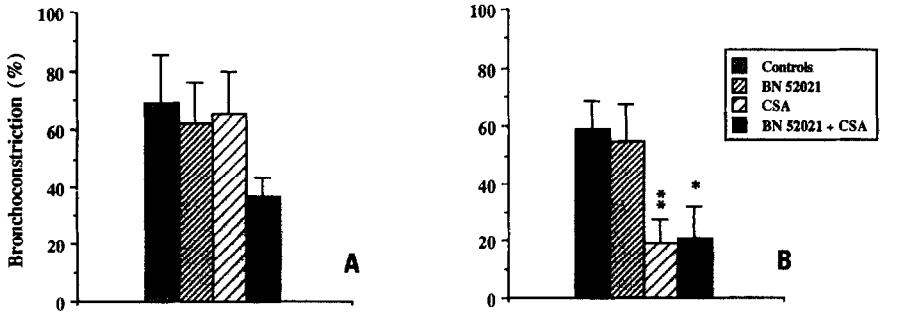

Fig.1: Effects of acute (A) and 2-day (B) treatment with CsA and BN 52021 either alone or in combination, on PAF-induced bronchoconstriction in the guinea-pig. The drugs CsA, BN 52021 or CsA and BN 52021 were given orally either 2 h or for 2 days before the intravenous injection of 60 ng/kg PAF (see Materials and Methods section for details). Values are means ± S.E.M. of at least 4 experiments. Statistical significativity was assessed by variance analysis, * : $p < 0.05$, ** : $p < 0.01$ and *** : $p < 0.001$ vs untreated animals.

CsA given alone for 48 hrs did not influence PAF-induced bronchoconstriction whereas pretreatment of the guinea-pigs with BN 52021 produced a partial decrease of the bronchopulmonary response (Fig. 1 B). This inhibitory effect of BN 52021 was not observed when acute treatment of the animals with this dose of the antagonist was performed (compare Fig. 1 A and Fig. 1 B). The association of BN 52021 with CsA doubled the inhibition with respect to that observed with BN 52021 alone (85 % vs 42 %, respectively) (Fig. 1 B).

Effect of treatment with CsA and BN 52021, given alone or in combination, on antigen-induced bronchoconstriction, leukopenia and thrombocytopenia in passively sensitized guinea-pigs.

Challenge of passively sensitized guinea-pigs with OA induced a bronchoconstriction (Fig. 2 A) accompanied by a marked decrease of the number of circulating leukocytes and without significant change in blood platelets counts (data not shown). Neither CsA nor BN 52021, given alone and as single treatment, prevented these phenomena to occur, whereas administered in association, they partially reduced the bronchocontrictor response by 46.1 % (NS) (Fig. 2 A), without effect on the antigen-induced leukopenia (data not shown).

The treatment of guinea-pigs for 2 days with CsA, but not with BN 52021, produced an inhibition of the antigen-induced bronchoconstriction of 68 % (Fig. 2 B). Treatment of the animals with both compounds produced an inhibition similar to that evoked by CsA alone.

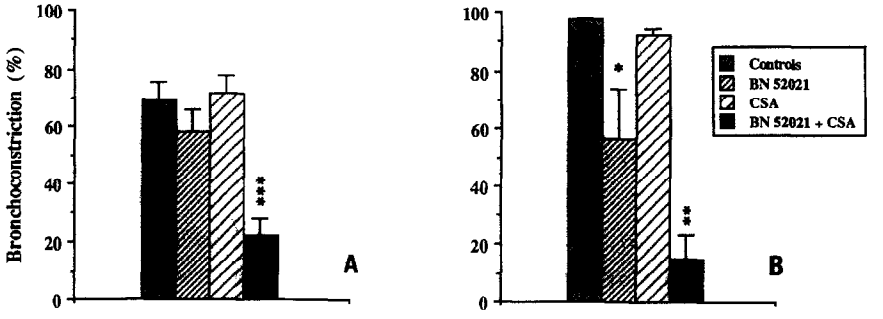

Fig.2: Effects of acute (A) and 2-day (B) treatment with CsA and BN 52021, either alone or in combination, on antigen-induced bronchoconstriction in the passively sensitized guinea-pig. The drugs CsA, BN 52021 or CsA and BN 52021 were given orally either 2h or for 2 days before the intravenous injection of 0.75 mg/kg ovalbumin (see Materials and Methods section for details). Values are means ± S.E.M. of at least 6 experiments. Statistical significativity was assessed by variance analysis, * : $p < 0.05$ and ** $p < 0.01$ vs untreated animals.

DISCUSSION

In accordance with previous reports (19-21), PAF induces in the guinea-pig a bronchoconstriction associated with a thrombocytopenia and a leukopenia. Although single administration of CsA alone has no effect on the PAF-induced bronchoconstriction, a marked inhibition of this phenomenon is observed when the drug is given along with an inactive dose of BN 52021. This effect of the association of the drugs on the bronchoconstriction is also related to an action on the PAF-induced alterations in the number of leukocytes and platelets. In addition, administration of CsA for 48 hrs, which alone does not influence PAF-induced bronchoconstriction, markedly increases the inhibition evoked by BN 52021. In this latter case, the combination of the two drugs almost doubles the inhibition with respect to that observed with BN 52021 alone.

Although bolus administration of CsA has no effect on the antigen -induced bronchoconstriction, a marked inhibition is observed when the drug is given for 2 days. Since passive sensitization takes place after the administration of the drugs, one possibility is that CsA blocks the in vivo sensitization of the cells. However, for technical reasons, this hypothesis could not be investigated since the sensitization process is of short duration, preventing passive immunisation of the guinea-pigs to be performed prior to the administration of the immunosuppressive drug. Another possibility is that CsA affects cell composition in the lung tissue and particularly that in eosinophils. This hypothesis is currently investigated since it has been shown that CsA is able to prevent hypereosinophilia in cyclophosphamide-treated and actively sensitized rats (22). Whatever the mechanism(s) of action of CsA, it is probably different in the case of the antigen-induced bronchoconstriction as compared to the effects of the drugs when the PAF-induced one was investigated (see above). Indeed, in this case, no synergistic effect of CsA and BN 52021 is noted, making unlikely that the immunosuppressive drug increases the activity of the PAF antagonist.

CsA has been suggested to induce the generation of PAF both in vitro and in vivo (14, 15). Pirotzky et al (16) have suggested that the nephrotoxic effect of CsA is due to the production of PAF, a fact reinforced by the blockade of the renal alterations induced by the immunosuppressive drug by BN 52063 (a mixture of different PAF antagonists) (15). Whether or not CsA induces a low in vivo generation of PAF which possibly desensitizes the animals to further stimulation with this autacoid remains to be determined.

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