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Theophylline reduces pulmonary eosinophilia after various types of active anaphylactic shock in guinea-pigs

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Abstract—The action of theophylline was studied on the inflammatory reaction obtained in bronchoalveolar lavage fluid 24 h after an active anaphylactic shock had been induced by ovalbumin inhalation in conscious sensitized guinea-pigs. The compound was administered twice by intraperitoneal administration after the anaphylactic reaction at a dose of 50 mg kg^{-1}. When the guinea-pigs were sensitized by intramuscular injection of 30 mg kg^{-1} ovalbumin or by ovalbumin aerosol, theophylline reduced the number of eosinophils and mononuclear cells in the fluid. When animals were sensitized by intramuscular injection of 30 mg kg^{-1} ovalbumin mixed with Freund's complete adjuvant, treatment with the xanthine derivative decreased only the number of eosinophils. In the three models theophylline did not modify significantly the number of neutrophils. Thus theophylline always reduced pulmonary eosinophilia irrespective of the mode of sensitization used to induce anaphylactic shock.

Theophylline has been used in the treatment of asthma for many years and is of particular value in some subjects (Nassif et al 1981; Brenner et al 1988) but it is not clear how this drug works. In addition to its bronchodilating properties pharmacological studies in animals have shown that the xanthine derivative has some anti-inflammatory effects (Page 1987). With regard to the supposed role of inflammation in the pathogenesis of asthma, this anti-inflammatory action may be important. Therefore we have examined the action of theophylline on the pulmonary inflammation resulting from an anaphylactic shock in guinea-pigs. We have chosen to use three already described models where by changing conditions of sensitization, we obtained quantitative differences in pulmonary inflammation after an anaphylactic shock was induced by ovalbumin administered by aerosol (Tarayre et al 1990, 1991).

Materials and methods

Animals. Male Dunkin Hartley guinea-pigs, 250–270 g, were used. The animals were divided into groups of 14–24.

Aerosol generation. A conscious guinea-pig was placed in a circular glass enclosure of 8 L in volume. Aerosol was induced by a Jouan nebulizer producing 1–3 μm dry particles at a flow rate of 28 mL h^{-1}. The nebulizer was supplied with compressed air at a pressure of 1 bar. The aerosol solutions were prepared in 0·9% NaCl.

Methods of sensitization and challenge. At various times after sensitization to ovalbumin, the animals were challenged for 6 min by an antigen aerosol with a concentration necessary to induce 10–20% mortality. In each experiment a group of sensitized animals exposed for 6 min to an aerosol of 0·9% NaCl (saline) represented sham-challenged controls.

In the first experiment, guinea-pigs were sensitized by intramuscular injection (1 mL kg^{-1}) of 30 mg kg^{-1} ovalbumin (grade V, Sigma) dissolved in a 50/50 mixture (v/v) of Freund's complete adjuvant (Difco) in saline (Tarayre et al 1990). Three to six weeks after sensitization the animals were exposed to an aerosol of a 5% solution (w/v) of ovalbumin.

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In the second experiment, animals were sensitized by intramuscular injection of 30 mg kg^{-1} ovalbumin in saline. Challenge was carried out after 3–6 weeks by exposure of animals to an aerosol of a 0·25% ovalbumin solution (Tarayre et al 1991).

In the third experiment, guinea-pigs were sensitized by exposure twice for 3 min to an aerosol of a 1% ovalbumin solution at a seven day interval (Hutson et al 1988). Seven days after the second exposure, challenge was carried out by exposure to an aerosol of a 1% ovalbumin solution.

Treatment. Anhydrous theophylline (Sigma) was administered after the anaphylactic shock rather than before it in order to avoid an anti-inflammatory effect related to the inhibition of the anaphylactic shock due to the bronchodilating properties of the xanthine derivative. Theophylline was administered twice (5 min and 5 h after the end of the challenge aerosol exposure) at a dose of 50 mg kg^{-1} in 10 mL kg^{-1} of saline intraperitoneally. The experimental doses are approximately of the same size as those used in our laboratory to inhibit by 50% the appearance of convulsions and of the mortality induced by the inhalation of histamine or antigen during a passive anaphylactic shock. Control groups (sham-challenged controls, sham-treated) received an intraperitoneal injection of solvent in the same experimental conditions as the treated group.

Measurement of bronchial inflammation. The bronchial inflammation kinetics obtained after an anaphylactic shock in animals sensitized following the three methods has already been described (Tarayre et al 1990, 1991). In comparison with sham-challenged controls, the guinea-pigs having suffered an anaphylactic reaction show in the three cases as an increase in the number of eosinophils, neutrophils and mononuclear cells. Preliminary experiments have shown that in the three modes of sensitization the number of various classes of leucocyte in sham-challenged controls is not significantly different from that in non-sensitized animals challenged by isotonic NaCl aerosol exposure. Certain differences in the inflammatory reaction obtained are evidenced according to the sensitization method. During sensitization by intramuscular injection of 30 mg kg^{-1} ovalbumin there was a greater increase in the number of neutrophils and mononuclear cells, particularly when using Freund's complete adjuvant. In the study of the effect of theophylline, animals were killed (300 mg kg^{-1} sodium pentobarbitone, i.p.) 24 h after anaphylactic shock, since the greatest inflammation occurs at this time for the three models. Their trachea were then cannulated and bronchoalveolar lavage carried out twice with 5 mL of sterile saline. Five mL of saline was injected slowly (10 s). After a dwelling time of 10 s the syringe piston was pumped several times before withdrawal of the maximum quantity of the liquid. The same procedure was repeated with a further 5 mL of saline. On average, 70–80% of the fluid administered was recovered. The leucocytes of the fluid were counted with a Coulter Counter (Coultronics, model ZF). After spreading on a slide, fixation and staining with May–Grunwald–Giemsa stain, the numbers of mononuclear cells, neutrophils and eosinophils per mL of fluid were determined. In preliminary experiments we verified in normal guinea-pigs that

the concentration of the various types of leucocyte was independent of the lavage fluid volume injected (from 10 to 40 mL).

Statistical calculations. Bonferroni's test or the non-parametric Kruskal-Wallis and Wilcoxon tests were used.

Results

In the first experiment, where animals were sensitized by intramuscular injection of 30 mg kg^{-1} ovalbumin mixed with Freund's complete adjuvant, there were 14 guinea-pigs in the sham-challenged control group. After the anaphylactic shock, 17 animals in the sham-treated group and 17 animals in the theophylline-group survived. The bronchoalveolar lavage fluid recovery was respectively 75 ± 2, 70 ± 3 and $69 \pm 3\%$ (no significant difference betweeen groups). In these experimental conditions, the xanthine derivative reduced significantly the number of eosinophils in the fluid (number not significantly different from that of sham-challenged controls) (Fig. 1A).

When guinea-pigs were sensitized by intramuscular injection of 30 mg kg^{-1} ovalbumin there were 22 animals in the sham-challenged controls and the same number in the sham-treated and theophylline groups after the anaphylactic reaction. The fluid recovery after bronchoalveolar lavage was respectively 73 ± 3, 77 ± 1 and $77 \pm 1\%$ (no significant difference between groups). Theophylline decreased significantly the number of eosinophils and of mononuclears (no significant differences compared with the number of these cells obtained in the sham-challenged controls) (Fig. 1B).

In the third experiment where guinea-pigs were sensitized by aerosol exposure, there were 15 animals in the sham-challenged group, 17 and 16 in the sham-treated and treated-group, respectively, after the shock. We recovered 76 ± 2, 75 ± 2 and $79 \pm 1\%$ of the fluid injected in the 3 groups (no significant difference). Theophylline also reduced significantly the number of eosinophils and of mononuclears (no significant differences compared with the sham-challenged group) (Fig. 1C).

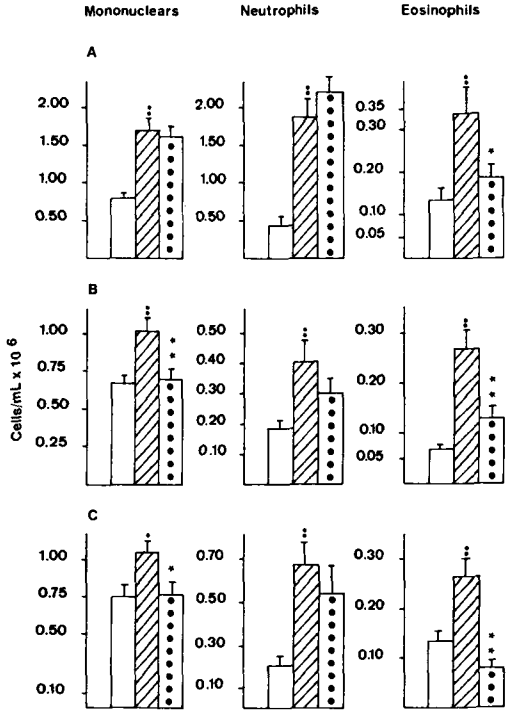

FIG. 1. Action of theophylline on the number of mononuclear cells, neutrophils and eosinophils in bronchoalveolar lavage fluid 24 h after an anaphylactic shock in guinea-pigs actively sensitized by various methods. A, Sensitization with 30 mg kg^{-1} (intramuscular) ovalbumin mixed with Freund's complete adjuvant (14–17 animals per group). B, Sensitization with 30 mg kg^{-1} (intramuscular) ovalbumin (22 animals per group). C, Sensitization by 2 exposures to ovalbumin aerosol (15–17 animals per group). □, Sham-challenged controls exposed to 0·9% NaCl aerosol challenge. ■, Sham-treated animals exposed to ovalbumin aerosol challenge. ⊡, Treated animals: 50 mg kg^{-1} of theophylline, 5 min and 5 h after challenge; vertical lines represent s.e. $^{\bullet}P < 0{\cdot}05$ $^{\bullet\bullet}P < 0{\cdot}01$ in comparison with sham-challenged controls, $^{*}P < 0{\cdot}05$ $^{**}P < 0{\cdot}01$ in comparison with sham-treated animals.

Discussion

The main result from this study was that independently of the mode of sensitization used to induce anaphylactic shock, theophylline always reduces pulmonary eosinophilia measured in bronchoalveolar lavage fluid. In addition, during sensitization by injection of a large dose of ovalbumin without Freund's complete adjuvant and during sensitization by an aerosol, theophylline decreases the number of mononuclear cells. On the other hand, in the three models, the xanthine derivative does not modify significantly the number of neutrophils in the lavage fluid.

In other guinea-pig models, it has been shown that theophylline reduced pulmonary eosinophilia after an anaphylactic shock induced according to a protocol of sensitization in favour of the IgE synthesis (Sanjar et al 1990) or after inhalation (Aoki et al 1988) or intraperitoneal injection (Sanjar et al 1989) of PAF-acether. Thus it is reasonable to suppose that theophylline acts upon mobilization of the eosinophils at the level of mechanisms common to immune and non-immune reactions. Clark et al (1977) have shown that at therapeutic concentrations aminophylline induced inhibition of eosinophil chemotaxis to endotoxin-activated serum.

Some effects of theophylline at the level of macrophages (Koopman et al 1973) and T lymphocytes (Bruserud 1984; Ilfeld et al 1985; Gillissen et al 1986; Scordamaglia et al 1988) have been described. Thus it is of interest that in two types of sensitization theophylline reduces the number of mononuclear cells in the bronchoalveolar lavage fluid. Further studies are necessary to explain the inactivity of the compound in the case of sensitization with ovalbumin mixed with Freund's complete adjuvant.

The relation between our results and the therapeutic action of theophylline in man is difficult to interpret. A decrease due to theophylline in pulmonary eosinophilia in allergic asthma has not been described. Ohman et al (1972) have shown a significant drop in the number of blood eosinophils after injection of aminophylline in healthy subjects. Even if the study of bronchial hyperreactivity and pulmonary eosinophilia has given rise to numerous contradictory results in animals and man (Sanjar et al 1990; Tarayre et al 1990, 1991; Holgate et al 1991) it is generally accepted that eosinophils are important cells in the inflammatory reaction of asthma. Bousquet et al (1990) have recently shown a correlation between the clinical severity of asthma and the number of eosinophils in the blood, in the bronchoalveolar lavage fluid and in the bronchial epithelium. The results we obtained in guinea-pigs underline the need to study thoroughly the effect of theophylline on the allergic inflammation in man and more particularly its action on eosinophil biology.

References

Aoki, S., Boubekeur, K., Sanjar, S. (1988) The effect of AH 21-132 and other anti-asthma drugs on PAF-induced eosinophilia in the guinea-pig lung. Br. J. Pharmacol. 93 (Suppl.): 54P

Brenner, M., Berkowitz, R., Marshall, N., Strunk, R. C. (1988) Need

for theophylline in severe steroid-requiring asthmatics. Clinical Allergy 18: 143–150

Bousquet, J., Chanez, P., Lacoste, J. Y., Barneon, G., Ghavanian, N., Enander, I., Venge, P., Ahlstedt, S., Simony-Lafontaine, J., Godard, P., Michel, F. B. (1990) Eosinophilic inflammation in asthma. New Engl. J. Med. 323: 1033–1039

Bruserud, O. (1984) The effect of theophyllamine on T-lymphocyte activation in vitro. Clin. Immunol. Immunopath. 32: 111–118

Clark, R. A. F., Sandler, J. A., Gallin, J. I., Kaplan, A. P. (1977) Histamine modulation of eosinophil migration. J. Immunol. 118: 137–145

Gillissen, G., Breuer-Werle, M., Ostendorp, H. (1986) The influence of theophylline treatment on PGE2 production, PGE2 sensitivity and the distribution pattern of lymphocyte classes relative to the immune status. Int. J. Immunopharmacol. 8: 561–568

Holgate, S. T., Roche, W. R., Church, M. K. (1991) The role of eosinophils in asthma. Am. Rev. Resp. Dis. 143 (Suppl. 3, part 2): S66–S67

Hutson, P. A., Church, M. K., Clay, T. P., Miller, P., Holgate, S. T. (1988) Early and late-phase bronchoconstriction after allergen challenge of non anaesthetized guinea pigs. I: the association of disordered airway physiology to leukocyte infiltration. Amer. Rev. Resp. Dis. 137: 548–557

Ilfeld, D., Kivity, S., Feierman, E., Topilsky, M., Kuperman, O. (1985) Effect of in vitro colchicine and oral theophylline on suppressor cell function of asthmatic patients. Clin. Exp. Immunol. 61: 360–367

Koopman, W.J., Gillis, M. H., David, J. R. (1973) Prevention of MIF activity by agents known to increase cellular cyclic AMP. J. Immunol. 110: 1609–1614

Nassif, E. G., Weinberger, M., Thompson, R., Huntley, W. (1981) The value of maintenance theophylline in steroid-dependent asthma. New Eng. J. Med. 304: 71–75

Ohman, J. L., Lawrence, M., Lowell, F. C. (1972) Effect of propranolol on the response of cortisol, isoproterenol and aminophylline. J. Allergy. Clin. Immunol. 50: 151–156

Page, C. P. (1987) Developments in Asthma. A View of Current Research. PJB Publications Ltd, Richmond, pp 17–20

Sanjar, S., Aoki, S., Boubekeur, L., Burrows, L., Coldite, I., Chapman, I., Morley, J. (1989) Inhibition of PAF-induced eosinophil accumulation in pulmonary airways of guinea pigs by anti-asthma drugs. Jap. J. Pharmacol. 51: 167–172

Sanjar, S., Aoki, S., Kristersson, A., Smith, D., Morley, J. (1990) Antigen challenge induces pulmonary airway eosinophil accumulation and airway hyperreactivity in sensitized guinea-pigs: the effect of anti-asthma drugs. Br. J. Pharmacol. 99: 679–686

Scordamaglia, A., Ciprandi, G., Ruffoni, S., Caria, M., Paolieri, F., Venuti, D., Canonica, G. W. (1988) Theophylline and the immune response: in vitro and in vivo effects. Clin. Immunol. Immunopath. 48: 238–246

Tarayre, J. P., Aliaga, M., Barbara, M., Tisseyre, N., Vieu, S., Tisne-Versailles, J. (1990) Model of bronchial hyperreactivity after active anaphylactic shock in conscious guinea pigs. J. Pharmacol. Methods 23: 13–19

Tarayre, J. P., Aliaga, M., Barbara, M., Malfetres, N., Vieu, S., Tisne-Versailles, J. (1991) Bronchial inflammation and hyperreactivity after an active anaphylactic shock in guinea pigs actively sensitized by systemic or aerosol route. Meth. Find. 13: 93–97

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The negative inotropic effect of diazepam in rat right ventricular strips

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Abstract—The effect of diazepam on cardiac contractility was investigated in electrically-driven right ventricular strips of the rat. Diazepam produced a concentration-dependent negative inotropic effect which was antagonized by either flumazenil, a benzodiazepine central-type receptor antagonist, or PK 11195, a benzodiazepine peripheral type receptor antagonist. The results suggest that the inhibitory effect of diazepam on cardiac contractility in the rat is mediated by both central and peripheral benzodiazepine receptors.

There are diverse reports on the effect of diazepam on cardiac contractility. Negative (Daniell 1975), positive (Castillo-Ferrando et al 1985) and biphasic (Gonzalez et al 1990) inotropic effects have been described. The mechanism involved in the inotropic actions of diazepam is also unclear. There is general agreement that diazepam acts by binding to specific receptors, which are mainly localized in the central nervous system. These central benzodiazepine receptors are coupled to the γ-aminobutyric acid (GABA) receptor and are linked to chloride channels. They are inhibited by the specific antagonist flumazenil or the convulsant agent picrotoxin (for review see Haefely et al 1985). Recently, a second class of diazepam binding site has been identified which is not coupled to the GABA receptor-chloride channel complex (Marangos et al 1982). These so-called peripheral-type receptors have been identified in kidney, heart and adrenals (Anholt et al 1985). It also appears that there are benzodiazepine receptors of the central-type in peripheral tissues (Luzzi et al 1986) as well as peripheral-type receptors in the brain (Benavides et al 1983). The non-benzodiazepine PK 11195 specifically binds to the peripheral sites and seems to antagonize the action of diazepam on these receptors (Mestre et al 1984).

The aim of the present study was to analyse the inotropic effect of diazepam and test for the involvement of central- and peripheral-type benzodiazepine receptors.

Materials and methods

Drugs. The drugs used were diazepam and flumazenil (a gift from Roche, Spain) and PK 11195 (1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide, generously supplied by Pharmuka Lab., Gennevilliers Cedex, France). These drugs were freshly dissolved in dimethylsulphoxide (DMSO obtained from Probus, Barcelona, Spain) and saline (4 DMSO:6 saline); this stock was diluted into prewarmed and preaerated bathing solution to achieve the desired final concentration.

Sprague-Dawley rats of either sex, 250–400 g, were stunned and exsanguinated. The chest was opened and the heart was rapidly removed and placed in Tyrode solution saturated with 95% O_2–5% CO_2 and the free wall of the right ventricle was excised. All procedures were performed in the presence of Tyrode solution of the following composition (mM): NaCl 136·9, KCl 5·0, $CaCl_2$ 1·8, $MgCl_2$ 1·5, NaH_2PO_4 0·4, $NaHCO_3$ 11·9, dextrose 5·0. Right ventricular strips were mounted longitudinally between two platinum electrodes under 1 g tension in Tyrode solution maintained at 34°C and gassed with 95% O_2–5% CO_2. The preparations were electrically stimulated (Grass