ANTI-ASTHMATIC ACTIVITY OF BB-1502 BY INHALATION

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Abstract—The anti-asthmatic activity, pulmonary mechanics and cardiovascular effects of BB-1502 (9-cyclohexyl-2-n-propoxy-9H-adenine) administered by aerosol were evaluated in guinea pigs and dogs and compared with the effects of salbutamol. BB-1502 protected guinea pigs from allergic asthma induced by aerosolized egg albumin antigen. The potency of BB-1502 was approx. 1/7 that of salbutamol when the test drugs and antigen were aerosolized simultaneously, whereas the two drugs were equipotent when administered 60 min prior to the antigen challenge. The duration of the anti-asthmatic action of BB-1502 appeared to be much longer than that of salbutamol. In dogs, BB-1502 and salbutamol showed comparable activity in preventing ascaris antigen-induced asthmatic symptoms. Disodium cromoglycate showed anti-asthmatic activity at high doses while aminophylline was inactive by inhalation. No cardio-pulmonary side effects were observed in guinea pigs or dogs following the inhalation of BB-1502. The doses were 800–5,000 times higher than those needed to produce the anti-asthmatic activity. Salbutamol caused significant tachycardia, hypotension and tachypnea at doses 100–300 times its anti-asthmatic dose.

The bronchodilator BB-1502 (9-cyclohexyl-2-n-propoxy-9H-adenine) is a member of a new chemical class and is active against allergic asthma in guinea pigs and dogs when administered by the oral or intraduodenal route (1). BB-1502, like aminophylline, is a potent inhibitor of cyclic AMP phosphodiesterase, but the cardiac and CNS stimulation produced by oral administration of BB-1502 was much less significant than that observed with aminophylline (1). Several β-adrenergic agonist bronchodilators have been used clinically by inhalation on the basis of their rapid onset of action and reduced side effects relative to those that may be evoked by systemic administration of the β-agonists. Although BB-1502 does not work through β-adrenergic receptor stimulation, its potent in vitro effectiveness in relaxing isolated tracheal smooth muscle (1) suggested its potential usefulness by inhalation as a topical bronchodilator. This paper describes the activity of BB-1502 when administered as an aerosol against allergic asthma elicited in guinea pigs and dogs. The anti-asthmatic activity of BB-1502 and its effects on pulmonary mechanics and the cardiovascular system were evaluated and compared with the effects of salbutamol, disodium cromoglycate and aminophylline.

MATERIALS AND METHODS

Egg albumin-induced anaphylaxis in guinea pigs: Anaphylactic asthma was
produced by an aerosol challenge of egg albumin (EA) in histamine-sensitive female guinea pigs (300–400 g) passively immunized with rabbit anti-EA serum as described previously (1). Each animal was placed in a 3-liter transparent glass chamber connected to a glass nebulizer (Nippon Shoji, Nisho type) and challenged with aerosolized antigen solution (2.5 mg EA/ml) for 10 min at a rate of 0.4 ml/min. Graded concentrations of the test compound solution were administered by inhalation simultaneously with or 60 min prior to the antigen challenge.

Ascaris antigen-induced asthma in dogs: The procedures used for the preparation of ascaris antigen (AA), the selection of AA-sensitive dogs by skin reaction, and the induction of anaphylactic asthma by AA aerosol have been previously described (1). Control and post-drug responses to the antigen aerosol were obtained at 2 hr intervals. Pulmonary resistance, tidal volume, and respiratory frequency were measured as pulmonary function parameters. Dynamic compliance was calculated from intraesophageal pressure and tidal volume. The intraesophageal pressure was determined by connecting a pressure transducer (San-ei Instrument, LPU-0.1) to a cuffed tube inserted into the esophagus. Nebulized test compound solutions were administered for 10 min at a rate of 0.2 ml/min immediately prior to the second antigen challenge. The anti-asthmatic activity of a test compound was assessed by comparing the pulmonary responses obtained after the first (control) and second challenges.

Cardiovascular studies in guinea pigs: Guinea pigs (500–600 g) of either sex were anesthetized and a cannula was inserted into the carotid artery 1 day prior to each experiment. The animals were individually placed into a 3-liter transparent glass chamber connected to a glass nebulizer. The test compound solution as an aerosol was introduced into the chamber for 15 min at a rate of 0.4 ml/min. Arterial blood pressure was measured by a pressure transducer (San-ei Instrument, LPU-0.5) through the cannulated carotid artery and the heart rate was recorded using a cardiotachometer (San-ei Instrument, type 2140), triggered by arterial pulses. Percentage changes in blood pressure and heart rate were calculated from the predrug control levels.

Cardiopulmonary studies in dogs: Anesthetized, spontaneously respiring mongrel dogs of either sex weighing 7–15 kg were used. Pulmonary function parameters were determined as described in the AA-induced asthma experiment. The test compounds were given as an aerosol for 10 min at a rate of 0.2 ml/min through a glass nebulizer connected to the branch of the endotracheal tube. Arterial blood pressure was measured by a pressure transducer and the heart rate was recorded by a cardiotachometer using the lead II attachment. Percentage changes in each parameter were calculated from the predrug control levels.

Drugs: The drugs used were aminophylline (Sigma Chemicals), BB-1502 (Bristol-Banyu Research Institute), disodium cromoglycate (Fujisawa), heparin sodium (Fluka AG), pentobarbital sodium (Ditman-Moor) and salbutamol hemisulfate (Sankyo). BB-1502, aminophylline, disodium cromoglycate and salbutamol were dissolved in 0.9% saline containing 10% ethanol and 0.8 or 2% tween 80. Other drugs were dissolved in 0.9% saline.

Statistics: EC50 values and 95% confidence limits (C.L.) were calculated by the method of least squares (2) in the dog asthma experiments and by the method of probit analysis (3) in the guinea-pig asthma experiments.

RESULTS

Effect on EA-induced asthma in guinea
pigs: BB-1502 and salbutamol showed dose-related inhibition of collapse induced by EA antigen challenge in guinea pigs. As shown in Table 1, the anti-asthmatic activity of BB-1502 was demonstrated over a dose range of 0.3 to 10 μg/ml (EC50=1.0 μg/ml) when the drug and antigen were administered simultaneously. Salbutamol was about 7 times more potent than BB-1502 (EC50=0.14 μg/ml), while inhaled aminophylline was inactive in this model. In an effort to assess the duration of drug effect, animals were treated with the test drugs by aerosol 60 min prior to the antigen challenge. As shown in the right hand column of Table 1, the EC50 values of BB-1502 and salbutamol obtained from the delayed challenge experiment were 9.6 μg/ml and 8.0 μg/ml, respectively. This indicates that the activities of both compounds were essentially equivalent at 60 min.

The time course of the anti-asthmatic activity of BB-1502 and salbutamol was further examined at equipotent doses which produced 80% protection of the anaphylactic asthma elicited by a simultaneous antigen challenge (EC80). The results are shown in Fig. 1. The maximal protective effect of BB-1502 was still present at 5 min, and substantial activity (>50% of the peak effect) could be demonstrated at 30 to 60 min after inhalation of the EC80 dose. In contrast, the activity of salbutamol diminished rapidly with 50% protection occurring after 5 min and no effect after 30 min.

Effect on AA-induced asthma in dogs:
The dogs pre-selected by the AA skin test exhibited a marked increase in pulmonary

| Compound          | Conc. (μg/ml) | P/T¹   | Conc. (μg/ml) | P/T²   |
|-------------------|--------------|--------|--------------|--------|
| Vehicle control   | —            | 3/28   | —            | 6/28   |
| BB-1502           | 10           | 11/12  | 60           | 8/10   |
|                   | 3            | 15/24  | 20           | 6/10   |
|                   | 1            | 12/24  | 6            | 9/20   |
|                   | 0.3          | 4/12   | 2            | 2/10   |
| EC50              | 1.0 (0.24~2.1) |       | 9.6 (3.1~31) |       |
| Salbutamol        | 1            | 8/10   | 80           | 7/8    |
|                   | 0.3          | 7/10   | 8            | 6/10   |
|                   | 0.1          | 4/10   | 4            | 4/12   |
|                   |              |        | 0.8          | 0/8    |
| EC50              | 0.14 (0.01~0.42) |       | 8.0 (3.1~25) |       |
| Aminophylline     | 10,000       | 2/10   | 3,000        | 1/10   |
|                   |              |        |              | ≈10,000|

¹ Number of animals protected/number of animals tested.
² 50% effective concentration in μg/ml with 95% C.I. in parenthesis.
Fig. 1. Comparative durations of the protective effects of aerosols of BB-1502 and salbutamol vs. EA-induced asthma in guinea pigs. The test drug doses employed were equiactive (80% protection) when administered simultaneously with the antigen challenge. Data show % protection of animals from collapse. Each point represents the results of at least 8 animals.

resistance and respiratory frequency and a decrease in tidal volume and dynamic compliance following the aerosolized AA challenge. As shown in Fig. 2, all of these pulmonary responses were reproduced in the same animals by a second AA challenge administered 2 hr after the first. Figure 3 demonstrates the protective effects of inhaled BB-1502 and salbutamol against AA-induced asthma. Control pulmonary responses upon AA challenge were determined in 4 animals per group. Graded doses of test compounds were administered by aerosol just before the second AA challenge. BB-1502 and salbutamol were similarly active, inhibiting the pulmonary responses markedly at 10 μg/ml, moderately at 1 μg/ml, but not significantly at 0.1 μg/ml. Table 2 summarizes the data of various pulmonary parameters examined. Aminophylline was inactive by inhalation at 1,000 μg/ml concentration, while disodium cromoglycate showed a significant activity in the dog model only at a very high concentration (EC50>10,000 μg/ml).

Cardiovascular effects in guinea pigs:
Inhalation of BB-1502 at 4,000 μg/ml produced a slight although not statistically significant tachycardia in conscious guinea pigs. No effects were seen on the heart rate and arterial blood pressure following aerosol administration of 2,000 μg/ml of BB-1502 (Table 3). In contrast, marked tachycardia occurred in guinea pigs treated with 3,000, 1,000, and 300 μg/ml of salbutamol. Some tachycardia was still evident at 30 μg/ml, the lowest dose tested.

Cardiopulmonary effects in dogs:
Table 4 shows the cardiopulmonary effects observed in anesthetized, spontaneously respiring dogs after administration of aerosolized BB-1502 and salbutamol. BB-1502 at 1,000 and 3,000 μg/ml did not produce any significant changes in the cardiopulmonary functions.
examined. To the contrary, inhalation of salbutamol caused a marked increase in respiratory frequency at 1,000 and 3,000 \( \mu \)g/ml, and the effect was still evident at 300 \( \mu \)g/ml. Salbutamol also produced a significant increase of heart rate at 1,000 and 3,000 \( \mu \)g/ml, and it produced significant decreases in blood pressure, pulmonary resistance, and tidal volume at the same dose levels.

**DISCUSSION**

BB-1502 given by inhalation was shown to be a potent inhibitor of asthmatic symptoms in experimental animals. In guinea pigs, salbutamol was 7 times more active than BB-1502 in relieving antigen-induced bronchoconstriction when test drug and antigen were administered simultaneously. However, the two drugs were equipotent...
Table 2. Protective effects of BB-1502, salbutamol, disodium cromoglycate and aminophyline on pulmonary reactions induced by ascaris antigen in dogs

| Compound                | Conc. (μg/ml) | N<sup>a</sup> | Pulmonary resistance | % protection (mean±S.E.)<sup>b</sup> | Tidal volume | Dynamic compliance | Respiratory frequency |
|-------------------------|--------------|---------------|----------------------|--------------------------------------|--------------|--------------------|----------------------|
| Vehicle control         | --           | 4             | -3±6                 | -11±9                                | -7±8         | -3±3               |                      |
| BB-1502                 | 10           | 4             | 81±7***              | 66±12**                              | 68±11**      | 85±6***            |                      |
|                         | 1            | 4             | 63±10**              | 28±7*                                | 27±5*        | 60±11***           |                      |
|                         | 0.1          | 4             | 0±17                 | 6±10                                 | 25±12        | 22±9*              |                      |
|                         |              |               |                      | EC50<sup>c</sup>: 1.4(0.58~4.0)      | 3.6(1.4~26)  | 3.7(1.2~16)        | 0.57(0.23~1.2)       |
| Salbutamol              | 10           | 4             | 78±6***              | 64±18*                               | 62±22*       | 86±10***           |                      |
|                         | 1            | 4             | 57±7***              | 47±8**                               | 32±6**       | 64±16**            |                      |
|                         | 0.1          | 4             | -1±4                 | -10±11                               | -6±8         | 3±13               |                      |
|                         |              |               |                      | EC50: 1.4(0.80~2.5)                   | 2.8(0.95~22) | 3.3(1.2~22)        | 0.95(0.31~12.8)      |
| Disodium cromoglycate   | 25,000       | 4             | 58±10**              | 48±3**                               | 44±8**       | 71±6***            |                      |
|                         | 2,500        | 4             | 18±4*                | 5±3                                  | 19±10        | 18±4**             |                      |
|                         |              |               |                      | EC50: 16,000(7,500~83,000)            | 25,000       | 25,000             | 10,000(6,700~16,000) |
| Aminophyline            | 1,000        | 4             | 17±9                 | 19±9                                 | 14±9         | 20±9               |                      |
|                         |              |               |                      | EC50: 1.000                          | 1.000        | 1.000              | 1.000               |

<sup>a</sup> N: number of dogs tested.

<sup>b</sup> Significantly different from control: *P < 0.05, **P < 0.01, ***P < 0.001.

<sup>c</sup> 50% effective concentration in μg/ml with 95% C.I. in parenthesis.
Table 3. Cardiovascular effects of BB-1502 and salbutamol after aerosol administration in conscious guinea pigs

| Compound        | Conc. (μg/ml) | N (a) | % change (mean±S.E.) (b) |
|-----------------|---------------|-------|--------------------------|
|                 |               |       | Heart rate | Blood pressure |
| Vehicle control | —             | 6     | 1±2         | -9±2          |
| BB-1502         | 4.000         | 6     | 7±4         | 3±2           |
|                 | 2.000         | 4     | 4±3         | 0±2           |
| Salbutamol      | 3.000         | 6     | 30±6**      | -11±2         |
|                 | 1.000         | 6     | 16±3**      | -8±1          |
|                 | 300           | 6     | 15±6*       | -12±1         |
|                 | 100           | 5     | 9±2*        | -12±3         |
|                 | 30            | 6     | 9±3*        | -8±2          |

(a): N: number of experimental animals.
(b): Significantly different from control: *P<0.05, **P<0.01.

Table 4. Cardiopulmonary effects of BB-1502 and salbutamol after aerosol administration in dogs

| Compound        | Conc. (μg/ml) | N (a) | % change (mean±S.E.) (b) |
|-----------------|---------------|-------|--------------------------|
|                 |               |       | Heart rate | Blood pressure | Pulmonary resistance | Tidal volume | Respiratory frequency |
| Vehicle control | —             | 4     | 0±2         | 2±3            | 1±5                  | 0±4         | -1±6                 |
| BB-1502         | 3.000         | 4     | 0±2         | -1±1           | -8±2                  | 6±3         | -5±3                 |
|                 | 1.000         | 4     | -3±2        | -2±1           | -7±3                  | -4±2        | -4±4                 |
| Salbutamol      | 3.000         | 4     | 17±2***     | -15±3**        | -22±5*                | -14±2*      | 125±16***            |
|                 | 1.000         | 4     | 13±3*       | -6±1*          | -15±5*                | -10±4*      | 47±8***              |
|                 | 300           | 4     | 9±4         | -5±2           | -9±2                  | -7±1        | 45±16*               |

(a): N: number of experimental animals.
(b): Significantly different from control: *P<0.05, **P<0.01, ***P<0.001.

when administered 60 min before the antigen challenge. This indicates that the duration of the action of BB-1502 is longer than salbutamol. This property of BB-1502 was also demonstrated in a time-course experiment in guinea pigs.

In the dog asthma model, BB-1502 and salbutamol were essentially equiactive as inhibitors of allergic bronchospasm induced by ascaris antigen inhalation. Disodium cromoglycate was active only at high doses in the dog model, while aminophylline was inactive when given by inhalation in both the guinea pig and the dog. As reported in the previous paper (1), BB-1502 relaxed isolated tracheal tissue at a concentration ca. 600 times lower than that required for aminophylline. This may partly explain the marked difference (>1,000 times) between the antiasthmatic activity of BB-1502 and aminophylline when given by inhalation, even though BB-1502 by the oral route was only 3-4 times more potent than aminophylline in experimental asthma studies (1). Anagnostopoulos and Komarek (4) reported that histamine-induced experimental asthma in guinea pigs was partially inhibited by aerosolized aminophylline at concentrations
as high as 10,000–30,000 $\mu$g/ml. A failure of aerosolized theophylline in a clinical trial has been reported by Stewart and Block (5). The level of activity shown by disodium cromoglycate in our experimental model confirmed the results reported by Yamatake, et al. (6).

It was reported in the previous paper (1) that cyclic AMP phosphodiesterase of guinea-pig lung origin was inhibited by BB-1502 (IC50: ca. 6.5 $\mu$g/ml) at approx. 15 times lower concentration than that required by aminophylline, and this inhibitory activity was postulated as one of the action mechanisms to explain the anti-asthmatic activity of BB-1502 following oral administration. In view of the potent anti-asthmatic activity of BB-1502 by inhalation (EC50: ca. 1.0 $\mu$g/ml) and the marked difference of inhalation activity between BB-1502 and aminophylline, there might still be other action mechanisms, in addition to the phosphodiesterase inhibition, involved in the anti-asthmatic activity of BB-1502.

Aerosol administration of BB-1502 at a concentration of 4,000 $\mu$g/ml did not produce significant tachycardia or hypertension in guinea pigs. This concentration was ca. 4,000 times the anti-asthmatic EC50 determined in the same species. To the contrary, aerosol administration of salbutamol did cause significant tachycardia and this effect was evident at concentrations ca. 200 times larger than the anti-asthmatic EC50 of salbutamol in the guinea pig.

Aerosols of a 3,000 $\mu$g/ml solution of BB-1502 did not produce any significant changes in cardiopulmonary functions in normal, anesthetized dogs. This concentration of BB-1502 was 800 to 5,000 times larger than the effective anti-asthmatic concentrations of the drug determined in ascaris antigen-challenged dogs. Aerosolized salbutamol did cause significant cardio-pulmonary responses in normal, anesthetized dogs including tachycardia, hypotension and increased respiratory rate. The latter effect occurred at concentrations of salbutamol approx. 100–300 times larger than the effective anti-asthmatic concentrations in dogs.

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