PHARMACOLOGICAL STUDIES ON BB-1502, A NEW BRONCHODILATOR

Hideo KAMEI, Minoru HIRANO, Kimio KAWANO, Shinji MURATA, Hideyo IMANISHI and Hiroshi KAWAGUCHI

Bristol-Banyu Research Institute, Ltd., Shimo-meguro, Meguro-ku, Tokyo 153, Japan

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Abstract—The pharmacological activities of BB-1502 (9-cyclohexyl-2-n-propoxy-9H-adenine), a potent bronchodilator and inhibitor of cyclic AMP phosphodiesterase, were compared with those of aminophylline in a number of biological systems. In vitro, BB-1502 relaxed guinea pig tracheal tissue at a concentration ca. 600 times lower than that required for aminophylline. The ability of BB-1502 to inhibit cyclic AMP phosphodiesterase of guinea pig lung origin was 15 times greater than that of aminophylline. The direct bronchodilator activity of BB-1502 determined in guinea pigs by the intraduodenal route was 5 times greater and the duration of the action longer than that of aminophylline. In dogs, intraduodenal BB-1502 inhibited bronchoconstriction induced by pilocarpine, histamine, or acetylcholine and the activity of the new compound was 4 to 7 times more potent than that of aminophylline. In experimental asthma studies, orally administered BB-1502 protected guinea pigs from allergy-induced asthma provoked by an antigen challenge with aerosolized egg albumin and the potency was 4 times greater than that of aminophylline. Ascaris antigen-induced asthma in dogs was completely inhibited by the oral administration of BB-1502 in a dose of 1 mg/kg with significant protection seen at 0.3 and 0.1 mg/kg. No complete protection was obtained with aminophylline in the dog asthma model. Cardiovascular effects observed with guinea pigs and dogs following the oral administration of BB-1502 were considerably less than those seen with aminophylline. CNS side effects were nil in rats when BB-1502 was given at 3 times the dose that produced significant stimulation by aminophylline. Acute lethal toxicity determined in mice by the oral route was similar for BB-1502 and aminophylline.

Aminophylline (theophylline ethylenediamine compound) has been widely used for the treatment of chronic asthma. This type of drug offers certain advantages over the sympathomimetic amines in that it has a longer duration of action and fewer side effects. However, problems associated with the use of aminophylline are the short half-life, narrow therapeutic range, and propensity to produce cardiovascular and CNS side effects (1, 2). Potent bronchodilators with a long duration of action and minimal side effects have long been awaited.

A series of 2,9-disubstituted adenine derivatives has been synthetized and tested in our laboratories in an effort to identify a potent, orally active bronchodilator with minimal side effects. Among these compounds, BB-1502 (9-cyclohexyl-2-n-propoxy-9H-adenine, Fig. 1) was selected.
for further study because of its promising pharmacological profile. BB-1502 is not a β-adrenergic stimulant but does resemble aminophylline in that inhibition of cyclic nucleotide phosphodiesterase is considered to be one of its major mechanisms of action. This paper describes the in vitro and in vivo pharmacology of BB-1502 as related to its bronchodilator activity as well as to its cardiovascular and CNS effects.

MATERIALS AND METHODS

Guinea pig tracheal preparation: Tracheas were excised from guinea pigs of either sex weighing 400 to 500 g. Tracheal chains were prepared according to the method described by Castillo and de Beer (3), except that the cartilage rings were cut, and suspended under 0.5 g tension in a 20-ml tissue bath containing Tyrode’s solution which was maintained at 37°C and aerated with a mixture of 95% O2 and 5% CO2. The relaxant effect of a test drug was expressed as a percentage of that obtained with 4×10⁻⁷ M isoproterenol. To check the β-adrenergic stimulant action of a test drug, its tracheal relaxant action was compared before and 5 min after treatment with a β-adrenergic blocking agent, propranolol (3.4×10⁻⁸ M).

Guinea pig atrial preparations: Right and left atria were excised from guinea pigs and suspended under 0.5 g tension in separate 20-ml tissue baths containing Krebs-Henseleit’s solution (37°C) aerated with 95% O2-5% CO2. The spontaneous contraction rate of right atria and the contractile force of electrically driven left atria were recorded. The electrical stimulation given to the left atria was in the form of square-wave pulses of 5 msec duration with voltages slightly above threshold (ca. 1 volt) at a frequency of 240 pulses/min.

Bronchodilator effect in guinea pigs: Pressure changes in a tracheal segment were measured in artificially ventilated guinea pigs, according to the method described by James (4). Guinea pigs of either sex weighing 500 to 600 g were anesthetized with a 30 mg/kg i.p. dose of pentobarbital sodium and a cannula was inserted into the lower trachea as closely as possible to the thorax for artificial ventilation. A rubber stopper was inserted into the lower end of the upper trachea. A second cannula filled with 0.9% saline was inserted into the upper trachea as far as possible from the lung to record intratracheal pressure on a polygraph (San-ei Instrument, type 142-8). The duodenum was cannulated for i.d. administration of the test compounds. The intratracheal pressure of the guinea pigs used for the experiment was approx. 50 mm H2O before drug administration. This decreased to a 50% level (ca. 25 mm H2O) after the i.v. administration of 0.03 μg/kg of salbutamol. Duration (half life) of bronchodilator action was determined by measuring the time from the onset to recovery to a 50% level of peak effect.
Inhibition of agonist-induced bronchoconstriction in dogs: Bronchial resistance was measured, according to the method of Konzett and Rössler (5). Mongrel dogs of either sex weighing 7 to 14 kg were anesthetized with pentobarbital sodium by an initial bolus injection of 30 mg/kg (i.p.) followed by i.v. infusion at 5 mg/kg/hr throughout the experiment. A cuffed endotracheal tube was inserted into the trachea for artificial ventilation and a positive inflow pressure was maintained at an approx. 100 mm H2O. Bronchoconstriction, as indicated by increased respiratory overflow pressure, was induced by the i.p. administration of pilocarpine hydrochloride at 0.5 mg/kg, according to the method of Giles et al. (6). After the resistance had reached a peak, the drugs were given i.v. in a cumulative mode and the bronchodilator effect estimated as a percent decrease in the pilocarpine-induced elevation of respiratory overflow pressure.

Bronchoconstriction was also induced by i.v. administration of histamine or acetylcholine in a dose necessary to give a 30% increase in bronchial resistance. Test compounds were administered through a catheter inserted into the duodenum.

Egg albumin (EA) antigen-induced anaphylaxis in guinea pigs: Anti-EA serum was prepared in rabbits, according to the method of Koda et al. (7) and used for anaphylactic asthma experiments in guinea pigs. Rabbits of either sex weighing 2.5 to 3 kg were immunized by an intradermal administration of EA (2 mg/rabbit) emulsified with complete Freund's adjuvant, followed by three booster injections of EA (1 mg/rabbit, i.m.) into a hind limb once weekly for 3 weeks. The animals were bled from the carotid artery 7 days after the last injection, and the antibody titer of the serum was determined by reversed single radial immunodiffusion assay (8). Serum samples showing a titer of over 1:8 were pooled, lyophilized and used for the experiments.

Female guinea pigs weighing 300 to 400 g were examined for their histamine sensitivity, and animals showing collapse within 2.5 min following inhalation of a 0.1% histamine solution were used for the experiment. The animals were passively sensitized by an i.v. administration of rabbit anti-EA serum (1 ml of 1:8 dilution of lyophilized antiserum). Eighteen hr after the sensitization, the animals were placed individually in a 3-liter transparent glass chamber connected to a glass nebulizer (Nippon Shoji, Nisho type) and challenged for 10 min with aerosolized antigen solution (4 ml, containing 2.5 mg EA/ml). The test compound was given to the animals orally 20 min prior to the challenge.

Ascaris antigen-induced asthma in dogs: Ascaris antigen (AA) was prepared as follows: Fresh swine worms, Ascaris suum, were homogenized and extracted with 5 mM phosphate buffer at pH 7.2. The extract was centrifuged (1,500xg, 20 min) and to the supernatant was added ammonium sulfate to a 60% saturation. The resulting precipitate was collected by centrifugation (10,000xg, 30 min) and dissolved in a minimum volume of the buffer solution. After dialyzation for 24 hr against the same buffer, the antigen solution was lyophilized and stored at 0°C. Mongrel dogs of either sex weighing 7 to 16 kg were anesthetized with pentobarbital sodium (30 mg/kg, i.p.) and given i.v. 0.5% Evans blue dye (0.2 ml/kg). Fifteen min later, 0.1 ml of graded concentrations of antigen solution (1 x 10^6 to 10^3 mg protein/ml) in 0.9% saline were given intradermally to elicit a skin reaction. Only the dogs which showed a positive reaction (obvious blueing of site after 30 min) with the antigen solution at concentrations equal to or less than 1 x 10^-2 mg protein/ml were selected for the experiment.

A cuffed tube with a branch connected to
a glass nebulizer was inserted into the trachea. According to the procedure described by Zimmermann et al. (9), the AA solution was administered twice by aerosol at a rate of 0.2 ml/min, for a 10 min period with a 3 hr interval between exposure, to first determine the control pulmonary response and then to evaluate the drug effect in the same animal during the second challenge. The concentration of antigen solution used for the challenge was adjusted to 1,000 times that of the lowest concentration producing a positive skin reaction. Respiratory flow was traced by a respiratory flow meter (Nihon-Kohden, MPF-1100) connected to an endotracheal tube inserted into the trachea and an alveolar pressure signal was obtained by closing the tube for about 0.2 sec. Tidal volume was obtained by electronic integration of the flow signal using an integrator (San-ei Instrument, type 1311A). Pulmonary resistance was calculated from the flow volume and alveolar pressure and respiratory frequency from the flow tracing. The response to the antigen challenge was expressed as a percent change from the control value obtained before the challenge. Test compounds were administered orally through an esophageal catheter 60 min 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: A cannula was inserted into the carotid artery of guinea pigs of either sex weighing 500 to 600 g, 1 day prior to the experiment. Arterial blood pressure was determined by connecting the cannula to a pressure transducer (San-ei Instrument, LPU-0.5) and the heart rate was recorded using a cardiotachometer (San-ei Instrument, type 2140). Graded doses of test compounds were administered orally and percent changes in blood pressure and heart rate were calculated from the predrug control levels.

Cardiopulmonary studies in dogs: Mongrel dogs of either sex weighing 7 to 15 kg were anesthetized by the procedure described in the bronchodilator studies. Pulmonary function parameters were determined by a respiratory flow meter (Nihon-Kohden, MPF-1100). Heart rate was determined by a cardiotachometer (San-ei Instrument, type 2140). Arterial blood pressure was measured by a pressure transducer (San-ei Instrument, MPU-0.5) through a cannulated femoral artery. Test compounds were administered orally through an esophageal catheter and percent changes in each parameter were calculated from the predrug control level.

Locomotor activity in rats: Groups of male Wistar rats weighing 200 to 350 g were given the test compounds orally. Fifteen min after drug administration, these rats were placed individually in a round activity cage (58 cm in diameter and 38 cm high, equipped with 4 photocell units) and their movements were counted automatically as they crossed the lightbeam.

Acute toxicity in mice: Acute toxicity was determined in male ddY mice weighing 20 to 25 g. Group of 10 animals were given the test compounds p.o., i.p. or i.v. Survivors or deaths were recorded daily for a period of 10 days to determine the median lethal dose (LD50).

Effects on low Km cyclic nucleotide phosphodiesterase: Lungs of 3 female guinea pigs weighing 400 to 450 g were homogenized with nine volumes of 40 mM Tris-HCl buffer (pH 7.4) containing 1 mM MgCl2. The homogenate was centrifuged (900×g, 20 min) at 0°C, and the supernatant fluid was used as a crude enzyme preparation. The phosphodiesterase (PDE) activity against cyclic AMP and cyclic GMP was assayed by the method of Thompson and Appleman (10) as modified by Boudreau and Drummond.
(11). The incubation medium (125 μl) contained 20 mM Tris-HCl buffer (pH 7.5), 7.5 mM MgCl₂, 12.5 μg bovine serum albumin, 2 μM U-³H-cyclic AMP (39.8 Ci/m mol., New England Nuclear) or 8-³H-cyclic GMP (21 Ci/m mol., Radiochemical Centre, Amersham), and 50 μl of enzyme preparation. The mixture was incubated at 30°C for 17-19 min to induce a 30% conversion of the substrate, and then boiled for 2 min to stop the reaction. After cooling at 30°C, 50 μl of Crotalus atrox venom solution (0.5 mg/ml. Sigma Chemicals) was added and the mixture incubated for 10 min at 30°C to convert the 5'-nucleotide formed to the nucleoside. The reaction was terminated with an addition of 0.5 ml of Dowex 1×8 resin which was pre-treated with 8.7 mM acetic acid and 118 mM formic acid for the assay of cyclic AMP and cyclic GMP PDE's, respectively. The mixture was shaken vigorously, allowed to stand for 10 min and centrifuged at 1,000×g for 5 min. A 0.2-ml portion of the supernatant was added to 8 ml of Bray's scintillation fluid and radioactivity determined by a liquid scintillation spectrometer (Packard, model 3375). Compounds tested for PDE inhibitory activity were dissolved in 20 mM Tris-HCl buffer (pH 7.5) containing 20% ethanol. A 25-μl volume of a test solution was added to the reaction mixture, with a similar amount of vehicle added to the controls.

Drugs: The drugs used are: acetylcholine chloride (Daichichi Chemicals), aminophylline (Sigma Chemicals), BB-1502 (Bristol-Banyu Research Institute), heparin sodium (Fluka AG), histamine diphosphate (Wako Pure Chemical Industries), dl-isoproterenol hydrochloride (Nakarai Chemicals), paraverine hydrochloride (Sigma Chemicals), pentobarbital sodium (Ditman-Moore), pilocarpine hydrochloride (Nakarai Chemicals) and salbutamol hemisulfate (Sankyo).

For in vitro and parenteral in vivo studies, BB-1502 was dissolved in 0.9% saline containing 5% ethanol and other drugs in 0.9% saline. For p.o. or i.d. administration, all drugs were dissolved or suspended in water containing 0.5% carboxymethyl cellulose.

Statistics: The EC20, EC50, IC50, ED20 and ED50 values and their 95% confidence limits (C.L.) were calculated by the method of least squares (12). The anaphylactic asthma experiment in guinea pigs was analyzed by the method of probit analysis (13). The LD50 and its 95% C.L. were calculated by the method of Litchfield and Wilcoxon (14).

RESULTS
Effects on isolated guinea pig trachea and atria: BB-1502 and aminophylline produced dose-related relaxations of guinea pig trachea. Table 1 shows a comparison between the EC50 values of both compounds determined before and after treatment with propranolol. The intrinsic potential of BB-1502 required to relax spontaneous tracheal tonus was markedly higher (ca. 600 times) than that of aminophylline. β-Adrenergic blockade with propranolol did not influence the relaxant activity of either of the test compounds.

The responses of guinea pig atria preparations to BB-1502 and aminophylline were opposite. BB-1502 showed negative inotropic and chronotropic effects, while aminophylline showed positive effects in both parameters (Table 1).

Bronchodilator effect in guinea pigs: BB-1502 and aminophylline produced a dose-related decrease in intratracheal pressure upon i.d. administration to anesthetized guinea pigs (Fig. 2). The ED50 values were 1.2 mg/kg for BB-1502 and 5.9 mg/kg for aminophylline. Figure 2 also shows the time course of bronchodilator action of BB-1502 and aminophylline determined with doses which elicited a near maximal effect. Over
a 90% decrease of intratracheal pressure was observed with 10 mg/kg of BB-1502 and 30 mg/kg of aminophylline. The half life of the bronchodilator action of BB-1502 (>120 min) was significantly longer than that of aminophylline (55 min).

Reversal of pilocarpine-induced bronchoconstriction in dogs: The administration of pilocarpine (0.5 mg/kg, i.p.) to anesthetized control dogs resulted in an increase of 52±5% (mean±S.E., N=8) in the respiratory overflow pressure. The i.v. administration of BB-1502 and aminophylline reversed the elevated pressure with doses over 0.1 and 0.3 mg/kg, respectively (Fig. 3). The ED50 values were 0.72 mg/kg for BB-1502 and 5.3 mg/kg for aminophylline.

Antagonism of histamine or acetylcholine-induced bronchospasm in dogs: The i.d. administration of BB-1502 or aminophylline produced a dose-related inhibition of histamine or acetylcholine-induced bronchospasm.

Table 1. Effects of BB-1502 and aminophylline on isolated guinea pig trachea and atria

| Compound | Trachea | Atria |
|----------|---------|-------|
|          | EC50a (10^-M) with 95% C.I. | EC20b (10^-M) with 95% C.I. | |
|          | Before propranolol | After propranolol | Inotropic (left atria) | Chronotropic (right atria) |
| BB-1502  | 0.095d | 0.072 | 62N | ca 620N |
|          | (0.081-0.110) | (0.055-0.092) | (51-69) | |
| Aminophylline | 66 | 64 | 40P | 210P |
|          | (54-81) | (52-78) | (29-82) | (140-380) |

a) The concentration required to induce tracheal smooth muscle relaxation equal to 50% of that obtained with 4×10^-7 M isoproterenol. b) The concentration required to induce 20% change from a control response. c) Determined 5 min after treatment with 3.4×10^-8 M propranolol. d) Values represent 4 to 6 experiments. e) N: negative effect. P: positive effect.

Fig. 2. Dose response (A) and duration (B) of bronchodilator effect of BB-1502 and aminophylline after i.d. administration in anesthetized guinea pigs. Each point represents the mean±S.E. of the peak responses obtained in at least 6 animals. a) The dose required to decrease the intratracheal pressure to a level equal to that induced by 0.03 µg/kg i.v. dose of salbutamol. b) Duration was the mean±S.E. of that of each animals, which was determined by the method as described in Materials and Methods.
in dogs, as shown in Fig. 4. The ED50 values against histamine-induced bronchoconstriction were 15 mg/kg for BB-1502 and 65 mg/kg for aminophylline. Against acetylcholine-induced bronchospasm, the ED50 of BB-1502 was 20 mg/kg and that of aminophylline more than 100 mg/kg. Comparison of respective ED50 values in these mediator-induced bronchospasms indicated BB-1502 to be at least 4 times more potent than aminophylline.

In the course of these experiments, two of six dogs treated with 100 mg/kg of aminophylline showed cardiac stimulation and abnormal respiration. Respiratory data for these 2 dogs were therefore excluded from the ED50 calculation. The dogs treated with BB-1502 (3 to 30 mg/kg, i.d.) showed no such cardiac and respiratory symptoms.

Effect on egg albumin antigen-induced asthma in guinea pigs: The passively immunized control animals all collapsed within 4 min after challenge with aerosolized EA antigen. The animals were protected from the anaphylaxis by oral pretreatment with BB-1502 or aminophylline, in a dose-related
fashion (Fig. 5). The oral ED50 values were 2.5 mg/kg for BB-1502 and 11 mg/kg for aminophylline.

Effect on ascariis antigen-induced asthma in dogs: The dogs pre-selected by the AA skin test showed marked pulmonary responses in the presence of an aerosolized AA challenge. In non-treated control dogs, an approx. 300% increase in respiratory frequency (f), 100% elevation of pulmonary resistance (PR) and 50~80% decrease of tidal volume (TV) were produced following the AA challenge. All of these pulmonary responses were reproduced in the same animals by a second AA challenge after a 3 hr interval. Figure 6 demonstrates the protective effects of BB-1502 and aminophylline in the AA-induced asthma model. Groups of animals (4 dogs/group) given the vehicle 1 hr before the first AA challenge to determine the control pulmonary responses and graded doses of test drugs were administered orally 1 hr before the second challenge. BB-1502 inhibited the pulmonary responses

| Compound  | Dose (mg/kg, p.o.) | N | % protection (mean±S.E.) |
|-----------|--------------------|---|--------------------------|
|           |                    |   | Pulmonary resistance     | Tidal volume | Respiratory frequency |
| Vehicle control |                   | 4 | -5±14                    | -3±7         | -10±11                  |
| BB-1502   | 1.0                | 4 | 91±7***                   | 97±2***      | 91±4***                  |
|           | 0.3                | 4 | 85±5***                   | 66±6***      | 88±5***                  |
|           | 0.1                | 4 | 63±14*                    | 46±17*       | 56±13**                  |
|           | 0.03               | 4 | 32±14                     | 22±16        | 31±12*                   |
| ED50<sup>a</sup> in mg/kg (95% C.L.) | 0.062              | 4 | 0.069                    | 0.069        |
| Aminophylline | 3.0                | 4 | -59±27                    | -5±15        | 7±18                     |
|           | 1.0                | 4 | 33±18                     | 20±8         | 32±19                    |
|           | 0.3                | 4 | 68±11**                   | 69±2***      | 85±4***                  |
|           | 0.1                | 4 | 23±7                      | 22±11        | 25±7*                    |

<sup>a</sup> number of experimental animals. <sup>b</sup> The dose required to protect the AA-induced pulmonary responses by 50%. Significantly different from control: *p<0.05, **p<0.01, ***p<0.001.
all but completely at 1 mg/kg, markedly at 0.3 mg/kg, moderately at 0.1 mg/kg but not significantly at 0.03 mg/kg. Aminophylline exhibited a significant inhibitory effect at 0.3 mg/kg but not at higher or lower doses. Table 2 summarizes the inhibitory effects of BB-1502 and aminophylline on various pulmonary reactions.

Cardiovascular effects in guinea pigs: Orally administered BB-1502 produced a decrease in heart rate in conscious guinea pigs and the dose-response curve was flat (Fig. 7). The peak effect observed with BB-1502 was a 17% decrease of heart rate at 100 mg/kg. In contrast, aminophylline produced tachycardia in a dose-related fashion, showing a 24% increase in heart rate at 30 mg/kg and a 28% rise at 100 mg/kg. Both compounds lowered arterial blood pressure but the effect was more pronounced with aminophylline (27% decrease at 100 mg/kg) than BB-1502 (12% decrease at 100 mg/kg).
kg) as shown in Fig. 7.

Cardiopulmonary effects in dogs: Figure 8 shows the cardiopulmonary effects observed in anesthetized dogs after the p.o. administration of graded doses of BB-1502 and aminophylline. Aminophylline caused a significant increase in heart rate at 10 and 30 mg/kg. In contrast to the bradycardia response demonstrated in the guinea pig experiment, BB-1502 showed a tendency to produce mild tachycardia in dogs at 30 mg/kg although the effect was not statistically significant. No significant hypotensive effect was noted with either compound up to the highest dose tested (30 mg/kg). About a 20% increase in respiratory frequency was observed with BB-1502 at 30 mg/kg, while aminophylline produced marked tachypnea at 10 mg/kg (78% increase) and 30 mg/kg (119% increase). Although data are not shown in Fig. 8, a reduction of pulmonary resistance was observed with BB-1502 (decreased by 8% at 3 mg/kg and 12% at 10 mg/kg), while a tendency toward increased resistance was noted with aminophylline (increased by 6% at 3 mg/kg and 12% at 10 mg/kg). No significant change in tidal volume was observed with either
compound at 10 mg/kg.

Effects on locomotor activity in rats: The CNS effects of BB-1502 and aminophylline were tested in rats using a locomotor activity cage and the results are shown in Fig. 9. The oral administration of BB-1502 had no effect on the locomotor activity of rats, up to a dose of 30 mg/kg. Aminophylline produced a mild but significant increase of locomotor activity at 10 mg/kg, and a more pronounced stimulatory effect at 30 mg/kg.

Acute toxicity in mice: The LD50 values obtained with BB-1502 and aminophylline given to mice by the i.v., i.p. or p.o. routes are shown in Table 3. These mice died within 24 hr. BB-1502 showed almost the same level of toxicity as aminophylline by the p.o. route but was more toxic than aminophylline when administered intraperitoneally.

Inhibition of low Km cyclic nucleotide phosphodiesterase: Figure 10 shows the results of dose-response experiments performed to determine the PDE inhibitory activities of BB-1502 and aminophylline. Concentrations of BB-1502 and aminophylline that inhibited the hydrolysis of cyclic AMP by 50% (IC50) were 23.6 and 350 μM, respectively, indicating approx. 15 times greater potency for BB-1502 than for aminophylline. Aminophylline inhibited cyclic AMP-PDE and cyclic GMP-PDE at similar concentrations. BB-1502 inhibited cyclic GMP-PDE at a concentration (144 μM).

![Fig. 9. Effect of BB-1502 and aminophylline on locomotor activity of rats by p.o. administration. Each point represents the mean ± S.E. of at least 7 animals. Significantly different from control: *p<0.05, **p<0.01, ***p<0.001.](image)

Table 3. Acute toxicity of BB-1502 and aminophylline in mice

| Compound       | LD50 in mg/kg |
|----------------|--------------|
|                | i.v.         | i.p.         | p.o.         |
| BB-1502        | >100*        | 170 (140-200)| 460 (410-520)|
| Aminophylline  | 220 (200-240)| 280 (250-310)| 420 (360-490)|

* not tested at higher doses because of insufficient solubility. Each value represents the mean with 95% C.I. of 10 male animals per dose level.
The concentration required to inhibit PDE activity by 50%.

**DISCUSSION**

BB-1502 was shown to have direct bronchodilator activity and potent antiasthmatic effects. In comparative evaluations with aminophylline in various animal models where drugs were administered by either the p.o. or i.d. routes, BB-1502 was 3 to 7 times more potent than aminophylline as a bronchodilator and as an inhibitor of antigen-induced anaphylactic asthma. The cardiovascular and CNS effects of BB-1502 were different in the type of adverse reactions or were less marked than those demonstrated by aminophylline.

In vitro, BB-1502 showed a much greater selectivity for tracheal smooth muscle than for cardiac muscle. The intrinsic potency of BB-1502 required to relax isolated trachea of guinea pigs was markedly higher than that of aminophylline. BB-1502 apparently does not involve β-adrenergic stimulation since tracheal relaxation was not blocked by propranolol. BB-1502 inhibited cyclic AMP-PDE prepared from guinea pig lung homogenate. The ability of BB-1502 to inhibit cyclic AMP-PDE was approx. 15 times greater than that of aminophylline on the basis of IC50 values. BB-1502 showed a greater selectivity for cyclic AMP-PDE than for cyclic GMP-PDE (ca. 6 times), while aminophylline inhibited the PDE's of both cyclic nucleotides at similar concentrations. The specific and marked inhibition of cyclic AMP-PDE by BB-1502 is considered to be one of the important action mechanisms involved in the selective relaxation of bronchial smooth muscle.

BB-1502, administered intraduodenally to anesthetized guinea pigs, produced a dose-related bronchodilation. The potency of BB-1502 was 5 times greater and the duration of action significantly longer than that of aminophylline. BB-1502 inhibited agonist-induced bronchoconstriction in dogs. Pilocarpine-induced bronchoconstriction was reversed by the i.v. administration of BB-1502 with 1/7 the dose required for aminophylline. The i.d. administration of BB-1502 antagonized histamine or acetylcholine-induced bronchospasm in dogs and was at least 4 times more potent than aminophylline.

In the experimental asthma model using guinea pigs passively sensitized with EA antiserum, oral pretreatment with BB-1502 protected the animals from collapse induced by an aerosolized EA antigen. BB-1502 was approx. 4 times more active than aminophylline in this model of allergic asthma. The antibody involved in this guinea pig asthma model has been reported to be IgG (15) and hence the model is somewhat different from clinical human asthma immunologically (15–17) and pathologically (18–20). Another asthmatic model employed
in the present studies was the ascaris antigen-induced asthma in spontaneously sensitized dogs. This model is considered to involve the reaginic antibody that resembles human IgE antibody seen in asthma in humans (21, 22). A dose-related inhibition of pulmonary reactions provoked by the antigen aerosol was demonstrated with the p.o. administration of BB-1502 in the dose range of 0.1 to 1 mg/kg with a complete inhibition seen at 1 mg/kg. In contrast, aminophylline showed a bell-shaped dose-response curve with significant activity demonstrated only at 0.3 mg/kg, but not at higher or lower doses, and no complete protection was obtained. The lack of activity at higher doses of aminophylline may partly be explained by the occurrence of tachypnea observed when a high dose of aminophylline was administered (Fig. 8).

The p.o. administration of BB-1502 caused a decrease of heart rate in guinea pigs but showed a tendency toward mild (insignificant) tachycardia in dogs at 30 mg/kg. The reason for this species-related difference in the heart rate response to BB-1502 is not known. Aminophylline produced significant tachycardia in both these species, with a dose of 10 mg/kg or higher. The hypotensive effect observed in guinea pigs was less significant with BB-1502 than with aminophylline. The p.o. administration of BB-1502 to dogs produced a mild increase (ca. 20%) in respiratory frequency at 30 mg/kg with no effect seen at lower doses. In a comparative experiment, aminophylline produced marked tachypnea (>70% increase) at 10 and 30 mg/kg. BB-1502 did not show any CNS side effects in the locomotion experiment in rats (10 to 30 mg/kg) while aminophylline produced significant locomotor stimulation at 10 and 30 mg/kg. Thus, BB-1502 appears to have fewer cardiovascular and CNS side effects than are seen with aminophylline.

The acute lethal toxicity determined in mice by the p.o. route was similar in terms of LD50 between BB-1502 and aminophylline.

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