Clinical and Experimental Pharmacology and Physiology (1990) **17**, 627–635

THE EFFECT OF INHALED FENOTEROL AND IPRATROPIUM BROMIDE ON PROPRANOLOL INDUCED BRONCHOCONSTRICTION IN THE ASTHMATIC AIRWAYS

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(Received 22 March 1990; revision received 9 May 1990)

SUMMARY

1. The provocative dose of inhaled propranolol, ($PC_{20}P$, mg/mL) needed to induce a 20% reduction in the forced expired volume in 1 s (FEV_1, L) was determined for 15 adult asthmatics following randomized pre-treatment with placebo, ipratropium bromide (40, 160 μg) and fenoterol (200, 800 μg) aerosols using a double-blind protocol.

2. Fenoterol 200 μg, 800 μg increased the baseline FEV_1 0.28 ± 0.16, 0.32 ± 0.16 L ($P = 0.04$, $P = 0.008$ respectively). Fenoterol 800 μg moved the $PC_{20}P$ rightwards from placebo geometric mean 10.95, 95% Confidence Intervals (95% CI) 4.43–27.22 mg/mL to mean 20.41, 95% CI 10.13 to 40.64 mg/mL ($P = 0.01$). Fenoterol 200 μg was not protective; mean PC_{20} 16.22, 95% CI 7.83–34.35 mg/mL ($P = 0.08$). Neither 40 or 160 μg ipratropium changed the FEV_1 or $PC_{20}P$ values compared with placebo; increase in FEV_1 0.15 ± 0.27 L ($P = 0.22$), 0.24 ± 0.12 L ($P = 0.14$) and geometric mean $PC_{20}P$ 16.59 ± 0.57 mg/mL, 95% CI 8.01–34.51 mg/mL ($P = 0.90$), 15.48 ± 0.66 mg/mL, 95% CI 6.72–36.05 mg/mL ($P = 0.34$) respectively after ipratropium treatments.

3. Bronchoconstriction induced by inhaled propranolol (P) appears to be only weakly antagonized by inhaled β-agonist and not reduced by antimuscarinic anticholinergic aerosol. This finding argues against the activation of a cholinergic reflex to explain propranolol induced bronchoconstriction (PIB).

Key words: asthma, β-adrenoceptor, bronchoconstriction induced by inhaled propranolol, cholinergic reflex, drug protection.

INTRODUCTION

Inhaled propranolol will induce bronchoconstriction in susceptible asthmatic airways (Langer 1967; Beumer 1968; Foresi *et al.* 1987; Okayama *et al.* 1987). Bronchoconstriction

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takes several minutes to fully develop and over 60 min to spontaneously revert and is associated with significant airways β-receptor blockade (Gerritsen *et al.* 1988; Ind *et al.* 1989). Pre-treatment with atropine and other antimuscarinic drugs or the use of these agents after induced bronchoconstriction, has been shown to offer protection against this airways effect and to expedite recovery from provoked bronchospasm in some asthmatics (Okayama *et al.* 1987; Gerritsen *et al.* 1988; Crimi *et al.* 1988; Ind *et al.* 1989). The claimed protective effect of anticholinergic agents suggests that propranolol provokes contraction of airway smooth muscle via a cholinergic pathway.

Propranolol can block prejunctional β_2-receptors present on cholinergic nerves and by this effect could exaggerate vagal tone by facilitating acetylcholine released (Rhoden *et al.* 1988; Barnes 1989). The expected increase in flow rates after exercise in normal subjects is inhibited by oral propranolol (80 mg) suggesting that the β-antagonist may exert two effects on airways calibre. First, direct bronchoconstriction via airways smooth muscle β_2-receptors and second, inhibition of β_2-receptors present on prejunctional cholinergic nerves resulting in vagally induced airways narrowing (Berkin *et al.* 1988). Although the degree of bronchodilatation with a β_2-agonist may be an indication of sensitivity to β-adrenoceptor antagonists, it is generally stated that bronchoconstriction by a non-selective β-blocker such as propranolol cannot be reversed by a β_2-agonist (Gayrard *et al.* 1975; Van Herwaarden 1983; Tattersfield 1986; Okayama *et al.* 1987). The protective effectiveness of large doses of inhaled β_2-agonist against bronchoconstriction induced by inhaled propranolol has not been rigorously tested. In this study, the role of the β-receptor has been contrasted with the importance of a cholinergic reflex in propranolol induced bronchoconstriction by comparing protection offered by aerosol fenoterol and ipratropium bromide given before inhaled propranolol. Both aerosol pre-treatments have been given in two doses to test for dose related protection.

METHODS AND MATERIALS

Fifteen adult asthmatics (Table 1) with stable symptoms were recruited from the outpatient clinics of Flinders Medical Centre. The study was approved by the Clinical Investigation Committee and each participant gave written informed consent. Each subject was shown to have bronchoconstriction induced by inhaled propranolol prior to entry into this study.

There were 13 men, mean age for the group 47 ± 15 (range 19–68) years. Thirteen of the subjects were atopic with two or more positive weal responses to skin prick testing with 12 common aero-allergens. Three subjects used no daily medication for control of asthma, 12 used a combination of inhaled β_2-agonist and beclomethasone dipropionate and in six, the inhaled medications were combined with ingested slow release theophylline. No subject used ipratropium bromide, sodium cromoglycate or antihistamine as a medication for the control of asthma. All subjects were studied at a time when symptoms were stable and there had been no exacerbation or medication change for 6 weeks. The baseline FEV_1 for the first test ranged between 50 and 117% predicted normal in the group and there was $< 10\%$ variation in baseline FEV_1 between tests (Crapo *et al.* 1981).

Each subject attended the laboratory on five separate test days at the same time of day. Treatments were randomly allocated, given double-blind, double-dummy and placebo-controlled. The FEV_1 was measured in triplicate using a single breath bellows spirometer (Vitalograph) both before, and at 15 min intervals for 45 min after delivery of aerosols. Aerosols were given to achieve the following effective treatments: (1) ipratropium bromide,

40 μg (IB40); (2) ipratropium bromide, 160 μg (IB160); (3) fenoterol, 200 μg (F200); (4) fenoterol, 800μg (F800); (5) placebo, (Pl). All aerosols were delivered by the experimenter through a spacer device (Aerochamber) to minimize variation in lung deposition. Forty-five minutes after each treatment the propranolol challenge commenced.

Propranolol inhalation tests were performed by the continuous nebulization method (Cockcroft 1985). The subject first inhaled a control solution of saline for 2 min using tidal breathing with the face mask loosely applied and the nose clipped. Air at 9 L/min was driven through a volume of 4 mL to generate an output of 0.15 mL/min from the same Wright nebulizer, which was used for all tests. After the saline inhalation, FEV_1 was measured at 0.5, 1.5 min and the lowest satisfactorily performed measurement was chosen as baseline. Increasing concentrations of propranolol hydrochloride (0.125–120 mg/mL) were delivered in identical manner until a greater than 20% fall in the lowest post-saline FEV_1 had been induced. Following each inhalation of propranolol, FEV_1 was measured at 1.5 min and each minute thereafter until broncoconstriction eased. Two stock solutions of DL-propranolol hydrochloride dissolved in saline, 16 mg/mL (pH 6.0, osmolality 347 mol/kg) and 32 mg/mL (pH 5.5, osmolality 374 mmol/kg) were further diluted with 0.9% saline to achieve less concentrated doses. The duration of nebulization was increased to deliver propranolol doses in excess of 32 mg/mL (max 8 min for assumed delivery of 128 mg/mL). The provocative concentration of inhaled propranolol ($PC_{20}P$, mg/mL) causing a 20% fall in the lowest post-saline FEV_1 was derived from the non-cumulative log dose–response curve for each challenge.

Statistical analysis

Each subject in the study represents a homogeneous block within which each of the five treatments (one placebo, four active treatments) were contrasted. Multiple analysis of variance (MANOVA), with repeated measures, was applied to the FEV_1, before and 45 min

Table 1. Baseline anthropometric, clinical and functional measurements

Patient No.	Sex	Age (years)	Height (cm)	Atopy*	FEV_1 PRED (1,BTPS)†	Baseline FEV_1 (%pred)‡	$PC_{20}P$§ (mg/mL)	Treatment**
1	M	51	169	+	3.5	73.5±3.2	24.0	S1200, B1600, Th900
2	F	44	168	+	3.1	113.2±6.5	8.3	S800, B800
3	M	44	175	+	4.0	98.3±2.0	57.0	Nil
4	M	67	175	−	3.0	101.8±3.0	10.6	S900, B900, Th600
5	M	68	190	+	4.0	117.8±1.6	33.0	S800, B800
6	M	58	173	+	3.5	49.5±1.5	9.3	S800, B800 Th600
7	M	57	169	+	3.4	87.8±3.9	16.2	S600, B600
8	M	62	170	−	3.3	65.3±4.1	4.1	S400, B100
9	M	38	171	+	3.9	58.3±2.9	0.33	F1600, B1200, Th800
10	M	53	170	+	3.5	74.9±2.3	7.0	S400, B100, Th600
11	M	19	181	+	4.8	96.8±2.6	18.0	Nil
12	M	52	177	+	3.8	82.3±2.2	32.9	S800, B400
13	F	44	165	+	2.8	98.3±2.9	26.0	S200, B100
14	M	28	171	+	4.2	86.3±1.0	35.5	Nil
15	M	23	181	+	4.7	100.6±5.7	0.38	S800, B400, Th900

*Positive response indicates >2 mm weal with skin prick testing to two or more of 12 common aero-allergens.

†Forced expired volume in 1 s, predicted normal values derived from Crapo *et al.* 1981.

‡Mean and s.d. for five tests expressed as per cent predicted.

§Provocative dose of inhaled propranolol to induce a 20% fall in baseline FEV_1 derived from preliminary test.

**S = salbutamol, F = fenoterol, B = beclomethasone dipropionate, as μg inhaled/day, Th = slow release theophylline as mg ingested/day.

after the treatments and the associated $PC_{20}P$ values (Norusis 1986). The contrasts were placebo and all other treatments and placebo with individual active treatments. Natural log transformation was applied before analysis of $PC_{20}P$ values. Responsiveness to inhaled propranolol is expressed as the group geometric mean, s.d. and 95% Confidence Intervals, which are tabulated as antilog values except for s.d. which remains untransformed (Bulpitt 1987). Pearsson's correlation coefficient was determined for: (1) baseline FEV_1, per cent predicted and $PC_{20}P$ placebo, (2) the FEV_1 change (L) after F800 and ratio $PC_{20}P$ F800/$PC_{20}P$ placebo; (3) baseline FEV_1, per cent predicted and FEV_1 change (L) for the F800 treatment. $P<0.05$ was considered significant.

RESULTS

Baseline FEV_1 values differed within the group and four subjects (1, 6, 8 and 9) demonstrated airways obstruction (Table 1). Baseline FEV_1 did not differ between the five tests. The FEV_1 increased after both fenoterol treatments, but neither dose of ipratropium bromide achieved bronchodilatation. FEV_1 increased 0.28 ± 0.16, range 0.01–0.56 L from baseline after fenoterol 200 μg and 0.32 ± 0.16, range 0.03–0.66 L after fenoterol 800 μg ($P = 0.044$, $P = 0.008$, respectively, compared with placebo); 0.15 ± 0.21, range -0.10–0.45 L after ipratropium 40 μg ($P = 0.22$) and 0.24 ± 0.12, range 0.02–0.46 L after ipratropium 160 μg ($P = 0.14$) (Table 2). The bronchodilator change, after the larger fenoterol dose, was inversely but weakly related to the baseline FEV_1 ($r = -0.48$, $P<0.05$).

Responsiveness to $PC_{20}P$ placebo varied widely within the group and was not associated with the degree of airways obstruction. Subjects 9 and 15 would be classified as severely responsive, subjects 2, 6 and 10 as moderately responsive and all others as mildly responsive to inhaled propranolol when compared with larger asthmatic groups challenged in an identical manner in this laboratory (Table 1). There was no correlation between baseline FEV_1 and $PC_{20}P$ placebo test ($r = -0.28$, $P>0.05$). The contrast between $PC_{20}P$ values

Table 2. Pre- and post-treatment FEV_1 values and responsiveness to inhaled propranolol

	Placebo	Ipratropium bromide (40 μg)	Ipratropium bromide (160 μg)	Fenoterol (200 μg)	Fenoterol (800 μg)
Baseline	3.24	3.25	3.18	3.18	3.18
FEV_1*	0.89	0.97	0.92	0.93	0.95
	(1.68–4.70)	(1.65–5.11)	(1.78–4.72)	(1.70–4.71)	(1.71–4.73
Post-	3.23	3.39	3.42	3.45	3.51
treatment	0.85	0.97	0.89	0.93	0.88
FEV_1†	(1.74–4.70)	(1.57–5.16)	(1.95–4.89)	(1.94–4.97)	(1.97–4.98)
$PC_{20}P$‡	10.96	16.59	15.48	16.22	20.41
	0.71	0.57	0.66	0.58	0.54
	(4.43–27.22)	(8.01–34.51)	(6.72–36.05)	(7.83–34.35)	(10.13–40.64)

*FEV_1 expressed as mean, s.d. and range (1, BTPS).

†FEV_1 measurements 45 min after treatment.

‡Provocative dose of inhaled propranolol (mg/mL) to induce a 20% fall in baseline FEV_1, expressed as geometric mean, s.d. and 95% confidence intervals.

after placebo and all other treatments, demonstrated a difference ($P = 0.002$). Though the lower dose of β-agonist achieved minor bronchodilatation, fenoterol 200 μg did not offer effective protection against the effect of propranolol in these patients (Table 2). Fenoterol 800 μg shifted the $PC_{20}P$ rightwards; placebo geometric mean 10.96 ± 0.71, 95% CI 4.43–27.22 mg/mL to mean 20.41 ± 0.54, 95% CI 10.13–40.64 mg/mL after F800. ($P = 0.01$) compared with mean 16.22 ± 0.58, 95% CI 7.83–34.35 mg/mL after F200 ($P = 0.08$) (Fig. 1b, d). Though there was an association between propranolol responsiveness, expressed as $PC_{20}P$ placebo and the degree of fenoterol protection, expressed as the ratio $PC_{20}P$ F800/$PC_{20}P$ placebo ($r = -0.65$, $P<0.05$), this association is dominated by the protective influence of F800 in the most responsive subjects. The degree of bronchodilatation was not associated with the degree of fenoterol protection ($r = 0.18$, $P>0.05$). Neither dose of ipratropium was able to protect the airways against the bronchoconstrictor effect of the β-antagonist, propranolol; $PC_{20}P$ 16.59 ± 0.57, 95% CI 8.01–34.51 mg/mL after IB40 and 15.48 ± 0.66, 95% CI 6.72–36.05 mg/mL after IB160 ($P = 0.90$, $P = 0.33$ compared with placebo) (Table 2; Figs 1a, 2c).

DISCUSSION

In this study, only a large dose of the β_2-adrenergic agonist, fenoterol has been able to offer partial protection against the bronchoconstrictive effect of the non-selective β-antagonist, propranolol, while the antimuscarinic aerosol, ipratropium bromide had no effect. The magnitude of fenoterol protection is small and is influenced by the mild propranolol

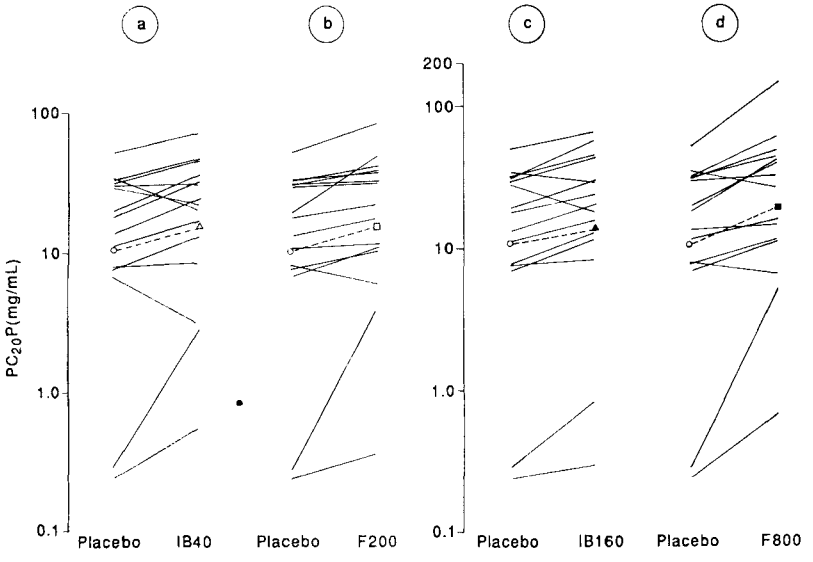

Fig. 1. Individual $PC_{20}P$ values after (a) placebo (0) and ipratropium bromide 40 μg (IB40)(△) and (b) placebo and fenoterol 200 μg (F200) (□) treatments. Individual $PC_{20}P$ values after (c) placebo (0) and ipratropium bromide 160 μg (IB160) (▲) and (d) placebo (0) and fenoterol 800 μg (F800) (■) treatments. The broken line indicates the change in geometric mean values.

responsiveness of these subjects. The group mean $PC_{20}P$, without treatment, is close to 10 mg/mL and the calculated propranolol dose deposited in the lower airways is 2.7 μmol, assuming 15% efficiency from a jet nebulizer (Lewis & Fleming 1985). By contrast, the estimated lower airways dose of the fenoterol (800 μg) is much smaller, 0.3 μmol and insufficient to compete on a molar basis with the antagonist. Systemic side effects, tachycardia and tremor restrict the maximal inhaled fenoterol dose to $\leq$1500 μg providing an approximate maximal lower airways dose of 0.73 μmol (Svedmyr 1985). Conditions for competitive interaction at the β-receptor are present in the most responsive of the subjects, 9 and 15 where the calculated airways dose of P would be 0.067 and 0.075 μmol respectively and the protective effect of F800 was three- and 20-fold.

The mechanism of propranolol induced bronchoconstriction (PIB) remains unclear, but anticholinergic drugs administered by the oral, intramuscular or inhaled route, are reported to antagonize PIB, whether the β-antagonist is inhaled or given intravenously (Gross & Skorodin 1984; Barnes & Thomson 1988). Antimuscarinic aerosols, ipratropium bromide (60 μg) in children, and oxitropium bromide (200 μg) in adults, accelerate recovery from PIB (Gerritsen *et al.* 1988; Ind *et al.* 1989). Inhaled atropine does not protect against $PC_{20}P$ in all asthmatics (Langer 1967; Okayama *et al.* 1987). In an early uncontrolled study, the degree of atropine protection against PIB was confounded by a large bronchodilator change from baseline, small sample of four asthmatics, and marked initial airways obstruction (Grieco & Pierson 1971). Okayama has identified marked intra-individual differences in the inhibitory effect of atropine on PIB in a group of 43 adults with asthma (Okayama *et al.* 1987). Nebulized atropine (total dose 6 mg) completely antagonized inhaled propranolol (maximum administered dose 13.2 mg) in 30 subjects, had a partial effect in three and failed to reverse bronchoconstriction in 10 subjects. The inability of atropine to overcome induced bronchoconstriction in these 10 subjects was matched by the failure of atropine pre-treatment to protect against PIB.

Inhaled vasoactive peptide (VIP) (100 μg) has no significant bronchodilator effect and offers mild protection against inhaled histamine in asthmatics (2.2-fold change in responsiveness) which is much less than the nine-fold change achieved by inhaled salbutamol (200 μg) (Barnes & Dixon 1984). Combined inhaled ipratropium bromide (40 μg) and VIP (70 μg) achieves a greater rightward shift (6.7-fold) in the $PC_{20}P$ than either agent alone (2 and 3-fold respectively). The mechanism for this greater than additive protection is unknown. The peptide may modulate acetylcholine release from postganglionic nerve terminals, clear released acetylcholine by changes in local blood flow or act directly on VIP receptors on airways smooth muscle (Crimi *et al.* 1988).

Ind *et al.* (1989) reported that inhaled oxitropium bromide (200 μg) fully protected against and completely reversed PIB, measured as a 35% fall in specific airways conductance (SGaw). Each of the seven asthmatics studied received a single predetermined dose of P, inducing between 17 and 64% reduction in SGaw but the exact rightward shift in responsiveness to P cannot be determined with this experimental design. It is difficult to separate the bronchodilator and protective effects of oxitropium; the $43 \pm 15\%$ improvement from baseline SGaw after oxitropium produced a large airways difference compared with placebo before inhaled P. In this same study, an isoprenaline bronchodilator dose–response after the final inhaled P dose, demonstrated that $\leq$100 μg of β-agonist could return the SGaw to baseline in three of the four asthmatics. These subjects had the most marked responsiveness to P and inhaled the lowest doses of the β-antagonist. This observation was in stark contrast to our experience that nebulized β-agonists produced only partial reversal of FEV_1 fall.

A direct comparison of cholinergic blockade of the asthmatic airways after oxitropium (200 μg) and ipratropium bromide (80 μg) has not been made but bronchodilatation measured by peak flow rates was similar (Peel *et al.* 1988). In separate studies, ipratropium (80 μg) achieved a 53-fold change in methacholine responsiveness (Bandouvakis *et al.* 1981) compared with a 129-fold change after treatment with oxitropium (200 μg) (Ind *et al.* 1989). The measured endpoint in the first study was a 20% change in FEV_1, and a 35% change in SGaw for the second. The site of propranolol effect in asthmatic airways is not established and the convention of regarding the two measurements as equivalent in tests of non-specific airways responsiveness may not apply to PIB (Cockcroft 1985; Macklem 1985). The PC_{20} FEV_1 may be less sensitive than PC_{35} SGaw so that higher concentrations of anticholinergic drug are needed to demonstrate blockade of PIB using this measurement. The lack of bronchodilatation and protection against PIB in our study suggests we are looking at a different population; inferior cholinergic blockade due to an inadequate dose of ipratropium or an inferior agent are unlikely given the controlled method of administration and the previously demonstrated efficacy of this antimuscarinic agent.

A defect in muscarinic autoreceptors (M_2) present on cholinergic nerves in the asthmatic airway has been proposed (Barnes 1989). In this model, blockade of β_2-receptors on postganglionic cholinergic nerves by P would leave acetylcholine release unchecked and promote contraction via M_3 receptors on airways smooth muscle (Barnes *et al.* 1988; Rhoden *et al.* 1988). Currently available antimuscarinic drugs, atropine, oxitropium and ipratropium bromide are non-selective anticholinergic agents that block both prejunctional (M_2) and postjunctional (M_3) muscarinic receptors with equal affinity and would therefore be expected to block the postulated (M_3) effect making the above hypothesis less likely. Testing of the hypothesis in the human airways awaits the development of selective postjunctional muscarinic (M_3) receptor antagonists. Inhaled salbutamol in conventional bronchodilator dose (200 μg) and larger doses (>1000 μg) consistently fails to reverse PIB (Gayrard *et al.* 1975; Okayama *et al.* 1987). In contrast, another study has shown low-dose isoprenaline can succeed if the propranolol airways dose is low (Ind *et al.* 1989). Direct comparisons of β-agonist and anticholinergic protection against PIB are rare and flawed by the parenteral or oral delivery of the β-agonist and the contrast with oxyphenonium bromide, a drug with both ganglion blocking and antimuscarinic properties (de Vries *et al.* 1982; Koeter *et al.* 1984).

β-Adrenergic agonists consistently fail to overcome PIB with any ease and in this study F800 offered only weak protection. The protective and the bronchodilator effects of a β-agonist cannot be separated but are only weakly linked. The prolonged duration of effect of propranolol in the airways and the failure of a competitive agonist to overcome bronchoconstriction suggests the involvement of secondary event(s) such as a conformational change in the β-receptor and/or mediator release. The ability of sodium cromoglycate to partially protect against PIB supports the involvement of preformed mediators (Koeter *et al.* 1982). β-Agonists can act as potent inhibitors of degranulation via β_2-receptors on mast cells and could play a modulating role if the cell contributes to PIB (Howarth *et al.* 1985). Most importantly, the study would not support the sole use of anticholinergic aerosols to treat bronchoconstriction induced by β-blocking drugs, but would recommend large doses of both anticholinergic and β-agonist aerosols, possibly combined with antagonists to preformed and secondary mediators. The usefulness of the latter combination against PIB needs to be tested in patients with moderate to marked responsiveness to propranolol.

ACKNOWLEDGEMENTS

The study was supported by a grant from the National Health & Medical Research Council of Australia. The authors thank ICI Australia and Protea Pharmaceuticals for the supply of propranolol powder and Boehringer Ingelheim Pty Ltd for provision of the canisters of fenoterol, ipratropium and placebo aerosols.

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