Comparative β -adrenoceptor blocking effects of propranolol, bisoprolol, atenolol, acebutolol and diacetolol on the human isolated bronchus

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Five β -adrenoceptor blockers, propranolol, acebutolol, diacetolol, atenolol and bisoprolol, were tested for their antagonistic effect against isoprenaline on human isolated bronchi. The results showed (1) that only propranolol exerted a competitive antagonistic effect against isoprenaline ($pA_2 = 9.40 \pm 0.22$, $n = 7$) whereas the other drugs did not, and (2) that, in the presence of β -adrenoceptor blockers in the plasma concentrations reported after a single usual therapeutic dose, the doses of isoprenaline giving the same bronchodilator effect must be multiplied by 32.6, 5.51, 4.63, 2.82 and 1.95 respectively with propranolol, diacetolol, acebutolol, atenolol, and bisoprolol. It was concluded that (1) atenolol and bisoprolol were the least potent drugs at bronchial level in therapeutic plasma concentrations and (2) that tests performed on the human isolated bronchus might be a useful screening procedure for new drugs with potential activity on the airways.

Keywords β -adrenoceptor blockers isoprenaline human bronchus

Introduction

β -adrenoceptor blockers are potentially harmful to patients with reversible airway obstruction. The evidence available suggests that β -adrenoceptor blockers which preferentially block cardiac β_1 -adrenoceptors are less likely to precipitate bronchospasm and therefore permit a relatively unhindered bronchodilatation with β_2 -adrenoceptor stimulants. There are, however, differences in the degree of selectivity of the cardioselective β -adrenoceptor blockers since they displace to varying extents the bronchial dose-response curves to β_2 -adrenoceptor stimulants. In clinical studies, atenolol or bisoprolol appeared to be more selective than other compounds, such as acebutolol, metoprolol or oxprenolol (Greefhorst & van Herwaarden, 1982; Gribbin *et al.*, 1981; Lammers *et al.*, 1984; Lawrence *et al.*, 1982; Macquin-Mavier *et al.*, 1988; Perks *et al.*, 1978; Ruffin *et al.*, 1979; Tattersfield *et al.*, 1984).

The development of simple screening procedures that enable this safety margin to be determined quantitatively at a pre-clinical stage is of considerable interest. In the present study, the effects of propranolol and of four cardioselective β -adrenoceptor blockers, atenolol, bisoprolol, acebutolol and diacetolol, against isoprenaline were compared on human isolated bronchi. The results thus obtained are in agreement with data from clinical trials and demonstrate that the human isolated bronchus can be used for preclinical studies.

Methods

Human bronchial tissue (usually with an inner diameter of 3 to 5 mm) was obtained from patients undergoing surgery for lung cancer but taken at a distance from the malignancy. Bronchi

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were dissected free of parenchyma and transported to the laboratory in ice-cold Krebs solutions previously aerated with a mixture of 95% O₂ and 5% CO₂. The tissue was stored overnight at 4° C, and the experiment was carried out on the next day.

Bronchial rings from a segmental bronchus were prepared and suspended in parallel in Krebs solution under an initial tension of 2.5 g. After 1.5 h of equilibration, the resting tension was between 1 and 2 g. Tensions were measured isometrically with strain gauges, amplifiers and I.O.S.-Moise 2 recorder system (Celaster, Celle l'Evescault, 86600 Lusignan, France) (Advenier *et al.*, 1986).

In all experiments, human bronchi were first contracted to maximal tension with acetylcholine (ACh), 3 mM, and then relaxed with theophylline, 3 mM.

After a 1.5 h rest period, the preparations were contracted with histamine 10⁻⁵ M to 70–90% of maximal contraction until a plateau was obtained. Concentration-response curves to isoprenaline were then established by cumulative addition in the absence or presence of incremental doses of β -adrenoceptor blockers. At the end of the concentration-response curve to isoprenaline, theophylline 3 mM was added to the bath to determine the 100% relaxation value. β -adrenoceptor blockers were added to the bath 30 min before the addition of histamine. Only one concentration of β -adrenoceptor blocker was used and one isoprenaline concentration-response curve was established for each bronchial ring.

The ratios of isoprenaline concentration in the absence or in the presence of β -adrenoceptor blocker in the plasma concentrations reported in the literature after a single usual therapeutic dose were calculated from the log (CR–1)/concentration of antagonist curves according to Arunlakshana & Schild (1959).

Results

Figure 1 shows the concentration-response curves to isoprenaline in the absence or presence of β -adrenoceptor blockers and the log (CR–1) vs concentration curves of β -adrenoceptor blockers assessed by the Arunlakshana & Schild method (1959).

In every case the curves were shifted to the right with preservation of maximal responses but only propranolol exerted a competitive antagonistic effect, since the regression slope of the log (CR–1)/antagonist concentration curve (0.82 ± 0.08) was not different from 1; pA₂ was $9.40 \pm$

0.22. With the other β -adrenoceptor blockers the regression slopes were significantly different from 1, so that their antagonistic effects could not be regarded as competitive (Figure 1).

The relative activities of isoprenaline in the absence or presence of β -adrenoceptor blockers calculated from the log (CR–1) vs concentrations of β -adrenoceptor blocker curves at concentrations equivalent to the plasma levels observed after administration of a usual therapeutic dose are shown on Table 1. It will be seen that in the presence of propranolol in therapeutic concentrations the dose of isoprenaline must be increased 32.6-fold to obtain the same relaxant effect. This increase was 5.51-fold with diacetolol, 4.63-fold with acebutolol, 2.82-fold with atenolol and 1.95-fold with bisoprolol.

Discussion

The potent bronchoconstrictor effect of propranolol in asthmatics has been evaluated quantitatively by Tattersfield *et al.* (1984) who have shown that the dose-response curves to isoprenaline are displaced 30-fold by a single 80 mg oral dose of propranolol, which agrees with our results (32.6-fold) (Table 1). When dose-response curves were established with salbutamol, the curves were more displaced than with isoprenaline, since the ratio for salbutamol doses in the presence or in the absence of propranolol was 21 (Gribbin *et al.*, 1979) or 30 to 50 (Mackay *et al.*, 1981) with a 40 mg dose of propranolol, or 61 with an 80 mg dose of propranolol (Gribbin *et al.*, 1979).

As regards acebutolol, our results show that the *in vitro* blockade of bronchial β -adrenoceptors it produces is 7 times less pronounced than with propranolol but 1.64 times more pronounced than with atenolol. Similarly Gribbin *et al.* (1981) found that after administration to asthmatics of acebutolol 200 mg, the displacement of the dose-response curves to salbutamol was respectively 2.01 and 2.87 more pronounced with acebutolol than with atenolol 50 mg and 100 mg.

The persistent partial β -adrenoceptor blocking effects of acebutolol, elicited by a decrease in bronchial response to salbutamol or terbutaline, has also been demonstrated in asthmatics or in smokers with chronic bronchitis who received 400 mg of acebutolol (Greefhorst & van Herwaarden, 1982; Macquin-Mavier *et al.*, 1988). In addition, Whitsett *et al.* (1982) have noted that in asthmatics who had received 800 mg of acebutolol per day for 5 days the effects of

Figure 1 Concentration-response curves to isoprenaline on human isolated bronchi in the absence or presence of β -adrenoceptor blockers in increasing concentrations. In the inset, are represented the $\log (CR-1)$ vs molar concentration of antagonist curves according to Arunlakshana & Schild (1959). Slopes of the regression curves were: 0.82 ± 0.08 ($n = 6$) for propranolol, 0.62 ± 0.09 ($n = 9$) for bisoprolol, 0.69 ± 0.12 ($n = 6$) for atenolol, 0.49 ± 0.06 ($n = 7$) for acebutolol and 0.55 ± 0.05 ($n = 7$) for diacetolol. pA_2 for propranolol was: 9.40 ± 0.22 ($n = 6$). Each point represents the mean $\pm$ s.e. mean ($n = 6$ to 9).

Table 1 Evaluation in healthy volunteers of plasma levels (free form) of different β -adrenoceptor blockers and calculation of the ratio of isoprenaline activity in the presence or absence of β -adrenoceptor blocker for this plasma level, from the log (CR-1)-antagonist concentration (Figure 1)

	Dose (mg) (single dose)	n	Plasma level C_{max} ($\mu\text{g ml}^{-1}$)	Protein binding ⁽⁵⁾	Plasma level (free form) (μM)	log (CR-1)	CR
Propranolol	80	12	$0.075 \pm 0.015^{(1)}$	90	0.0289	1.50	32.6
Bisoprolol	10	11	$0.051 \pm 0.004^{(2)}$	30	0.11	-0.02	1.95
Atenolol	100	6	$0.511 \pm 0.059^{(3)}$	5	1.82	0.26	2.82
Acebutolol	400	5	$0.735 \pm 0.090^{(4)}$	10	1.97	0.56	4.63
Diacetolol (Acebutolol)	400	5	$0.774 \pm 0.067^{(4)}$	5	2.39	0.65	5.51

References are: (1) Duchin *et al.* (1984), (2) Hayes *et al.* (1987), (3) Schäfer-Korting *et al.* (1983), (4) Roux *et al.* (1983), (5) Giudicelli & Witchitz (1983). CR = Concentration-ratio of isoprenaline in the presence or in the absence of β -adrenoceptor blocker.

terbutaline (5 mg orally) on FEV₁ were inhibited to the same extent as after treatment with propranolol 80 mg per day for 5 days.

The bronchial β -adrenoceptor blocking effects of diacetolol, an active metabolite of acebutolol (Thibonnier *et al.*, 1982), have never been studied in humans but they appeared, in our results, to be very similar to those of the parent-compound.

Our results show that among the β -adrenoceptor blockers tested atenolol and bisoprolol have the least activity on bronchi. When present in concentrations equivalent to therapeutic plasma levels, the doses of isoprenaline required for relaxation must be multiplied by 2.82 and 1.95 respectively. These two compounds thus appear to be 11.5 and 16.7 times respectively less active than propranolol on bronchi.

Concerning atenolol, Gribbin *et al.* (1981) studied eight asthma patients under salbutamol and found ratios of activity of 1.4 and 1.95 respectively with atenolol 40 and 80 mg. These results are comparable to our projections, in

agreement with the good tolerance of this drug (see introduction).

As for bisoprolol, Tattersfield *et al.* (1984) conducted clinical trials in asthmatics and reported isoprenaline dose ratios of 1.04 and 3.04 respectively with bisoprolol 20 and 40 mg. This high safety margin has also been emphasized by Macquin-Mavier *et al.* (1988), who showed that, contrary to acebutolol 400 mg, bisoprolol in doses of 10 mg has no effect on the dose-response curves of salbutamol in smokers with chronic bronchitis in agreement with Lammers *et al.* (1984), Dorow & Tönnemann (1984) and Dorow *et al.* (1986).

To conclude, it would appear from the present study that atenolol and bisoprolol in therapeutic plasma concentrations are the least potent drugs at bronchial level; this is in agreement with published clinical trials. Furthermore tests performed on the human isolated bronchus might be a useful *in vitro* screening procedure for new drugs with potential activity on the airways.

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