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The effects of indomethacin on the refractory period to hypertonic saline-induced bronchoconstriction

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The effects of indomethacin on the refractory period to hypertonic saline-induced bronchoconstriction. R.J. Hawksworth, S.P. O'Hickey, T.H. Lee.
ABSTRACT: The purpose of this study was to determine the effect of pretreatment with indomethacin on the refractory period to hypertonic saline-induced bronchoconstriction. In a double-blind, placebo-controlled, randomized trial nine asthmatic subjects underwent two hypertonic saline challenges, 60 min apart, on a control day and after premedication with indomethacin 50 mg or matching placebo, twice daily for three days.

Premedication with indomethacin did not change airways responsiveness to the initial hypertonic saline challenge. The mean maximal % fall in specific airway conductance (sGaw) was 40.3, 44.1 and 47.6% on the control, placebo and indomethacin days, respectively. Subjects were significantly less responsive to the second hypertonic challenge as compared to the initial challenge on all three study days. There was a variable effect of indomethacin pretreatment on the refractory period. Five subjects lost their refractory period after indomethacin, when the variability of the test was taken into account. This suggests that there may be contributory mechanisms to the refractory period other than the release of protective prostanoid metabolites.
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Inhalation of hypertonic saline aerosol induces bronchoconstriction in many asthmatic patients [1]. In some subjects, bronchoconstriction is followed by the development of a refractory period during which a second hypertonic saline challenge will elicit significantly less bronchoconstriction [2]. The pathogenesis of refractory behaviour has not been determined.

Previous studies have suggested that refractory behaviour to hypertonic saline challenge cannot be attributed to protective catecholamines [3], a reduction of nonspecific airways responsiveness after the initial challenge [4], or mediator depletion of airway mast cells [3, 5]. Refractory behaviour also occurs after exercise-induced asthma [6], ultrasonically nebulized distilled water (UNDW) challenge [1, 7], and dry-air hyperventilation [8]. Refractoriness to exercise [9] and UNDW [10] can be attenuated by the pre-administration of indomethacin. This suggests that cyclo-oxygenase products may be responsible for the development of the refractory period. We have investigated the effects of indomethacin premedication on the airways responsiveness and refractoriness to hypertonic saline in a group of nine asthmatic subjects.

Methods

Subjects and study design

Nine atopic asthmatic subjects (4 male, 5 female) aged 18–37 yrs (mean 23 yrs) were studied. The clinical details are given in table 1. Atopy was defined as a positive response (a wheal of ≥3 mm greater than the control solution) to a range of common aeroallergens (grass pollen, cat fur, dog hair, *D. pteronyssinus*).

Table 1. – Clinical details of the subjects studied

Subject no.	Age yrs	Sex	Treatment	Ht m	FEV_1 % pred
1	30	M	S	1.7	100
2	18	F	S, BDP	1.7	95
3	37	M	S	1.9	100
4	26	F	S, BDP	1.6	93
5	21	M	S	1.7	80
6	19	F	S	1.6	91
7	19	M	S	1.8	87
8	22	F	S	1.5	85
9	19	F	S	1.7	83

S: salbutamol, 200 μg, given as needed; BDP: beclomethasone dipropionate, 200 μg *b.d.*

Study design

Subjects attended the laboratory on three separate occasions. On the initial visit the subjects underwent two hypertonic saline challenges (HS1, HS2) one hour apart. Subsequently, the subjects underwent identical hypertonic saline challenges on two occasions, which were separated by two weeks. Each of these subsequent challenge days was preceded by the administration of 50 mg indomethacin or matching placebo, twice daily, for three days prior to challenge, in a

double-blind manner. For each individual, all challenges were performed at the same time of day and the volumes of hypertonic saline given on the two study days were identical to those given on the initial control day. All subjects gave informed consent and the study was approved by Guy's Hospital Ethics Committee.

Hypertonic saline challenges

Hypertonic saline challenges were performed as described previously [4]. On the first study day, subjects inhaled doubling volumes of hypertonic (3.6%) saline whilst seated in a total body plethysmograph. Airway calibre was determined by changes in specific airways conductance (sGaw) at 30 s intervals for 5 min after each dose and the challenge was continued until a 35% fall in sGaw was achieved, or a maximum dose of 315 *l* of aerosolised hypertonic saline was administered. On subsequent study days, an identical procedure was followed and the same cumulative dose of hypertonic saline was given. The maximal % fall in sGaw invariably occurred within 5 min and this value was used for analysis.

Analysis of data

Differences in the maximal % fall in sGaw between HS1 and HS2 on each study day and % fall after HS1 between each of the study days were analysed using one way analysis of variance with replications. The coefficients of variation for maximal % fall in sGaw after HS1 on the control and placebo days were derived from the standard deviation of the values expressed as a percentage of the overall mean. A change of greater than twice the coefficient of variation was considered to be significant.

Table 2. – Baseline sGaw values ($s^{-1} \cdot kPa^{-1}$) for each subject prior to each airway challenge

Subject	Control		Placebo		Indomethacin	
no.	HS1	HS2	HS1	HS2	HS1	HS2
1	2.08	1.87	1.84	1.87	1.85	2.10
2	2.14	1.98	2.84	2.47	2.57	2.46
3	2.00	1.76	1.97	2.34	1.37	1.12
4	2.23	2.63	1.89	2.17	1.91	2.23
5	1.49	1.31	1.52	1.44	1.45	1.41
6	3.59	2.85	2.92	2.50	2.73	2.49
7	1.83	1.68	1.52	1.34	2.12	2.00
8	1.34	1.55	1.21	1.06	0.92	0.87
9	1.97	2.36	2.36	2.44	2.37	2.42
Mean	2.07	1.99	2.01	1.96	1.92	1.90

HS1: initial hypertonic saline challenge; HS2: hypertonic saline challenge 60 min later.

Results

There was no significant difference between the baseline sGaw values on any of the study days (table 2).

On the control day, the mean maximal % fall in sGaw after HS1 was 40.3% (range 34–57%) and after HS2 was 21.7% (range 7–31%), p<0.0001. After premedication with placebo, the mean maximal % fall in sGaw after HS1 was 44.1% (range 20–55%) and after HS2 was 27.3% (range 16–38%), p=0.002 (table 3). After premedication with indomethacin, the mean maximal % fall in sGaw after HS1 was 47.6% (range 32–64%) and after HS2 was 32.9% (range 10–50%), p=0.01 (table 3).

The mean % reduction in falls in sGaw between HS1 and HS2 on the control, placebo and indomethacin days were 51.7, 33.7 and 31.9%, respectively.

Table 3. – Maximal airway response to two hypertonic saline challenges, on the control day and after premedication with placebo and indomethacin 50 mg twice daily for three days

Subject	Control			Placebo			Indomethacin		
no.	HS1	HS2	%	HS1	HS2	%	HS1	HS2	%
1	43	30	30	52	38	27	48	41	15
2	42	17	60	54	23	57	43	50	-16
3	38	7	82	43	29	33	45	20	56
4	57	31	46	55	33	40	53	50	6
5	34	20	41	20	24	-20	32	16	50
6	42	25	40	33	16	52	40	10	75
7	54	20	63	54	29	46	51	36	29
8	43	27	37	50	25	50	52	38	27
9	53	18	66	36	29	19	64	35	45
Mean	40.3	21.7	51.7	44.1	27.3	33.7	47.6	32.9	31.9
	p<0.0001			p<0.0001			p<0.02		

HS1: maximal % fall in sGaw after initial hypertonic saline challenge; HS2 maximal % fall in sGaw after a second hypertonic saline challenge 60 min later; %: % reduction in sGaw between HS1 and HS2, calculated as [HS1-HS2/HS1] ×100. Probability values are for differences between HS1 and HS2 on each study day as analysed by one way analysis of variance with replications. sGaw: specific airway conductance.

These changes were not significantly different from each other (p=0.190). The coefficient of variation for HS1 on the control and placebo days was 14.7%. If refractoriness is defined as a % reduction of equal or greater than twice the coefficient of variation (≥30%) of repeated HS challenges, all subjects were refractory on the control day. On the placebo day, subjects nos 2, 3, 4, 6, 7 and 8 had refractoriness and on the indomethacin day, subjects nos 3, 5, 6 and 9 were refractory. Thus, five subjects (Nos 1, 2, 4, 7 and 8) lost their refractoriness after indomethacin treatment.

Discussion

The results of this study demonstrate that premedication with indomethacin did not alter airways responsiveness to an initial hypertonic saline challenge. The dose of indomethacin used in this study has been shown to inhibit prostaglandin (PG) synthesis [11, 12]. The failure of indomethacin to inhibit hyperosmolar saline responsiveness suggests that bronchoconstrictor prostaglandins such as PGD_2, $PGF_{2\alpha}$ and thromboxane A_2 may not be responsible for hypertonic saline-induced bronchoconstriction. This present finding is different from that of FINNERTY *et al.* [13]. They demonstrated that pretreatment with flurbiprofen, which is 20 times more potent than indomethacin [14] in inhibiting microsomal cyclo-oxygenase, attenuated airways responsiveness to hypertonic saline challenge when the hyperosmolar saline was administered in a cumulative dose-dependent manner. The same study [13] demonstrated no effect of flurbiprofen on airways responsiveness to hyperosmolar saline when this was administered as a single bronchoconstricting dose. Thus, the difference between our present findings and those of the previous study may relate to differences in study design or to the differing potencies of cyclo-oxygenase inhibitors used.

The present results also demonstrate that premedication with indomethacin abolished refractoriness in only five of the subjects studied. We have defined refractoriness as twice the coefficient of variation of hypertonic saline challenge. Since the variation in falls in sGaw was 15%, we have considered a ≥30% decrease in sGaw between HS1 and HS2 as refractoriness. The heterogeneity of response to indomethacin was unlikely to have been due to poor compliance of the subjects with their medication, although this cannot be excluded. Previous studies of the effects of indomethacin on refractoriness to other airway challenges have demonstrated similar results. MARGOLSKEE *et al.* [15] investigated the effects of indomethacin, 25 mg 4 times a day, for seven days, on refractoriness to exercise and eucapnic hyperventilation. Pretreatment with indomethacin abolished refractory behaviour to exercise in the group as a whole. However, in the seven subjects studied, two subjects still demonstrated refractoriness after premedication with indomethacin. Indomethacin did not abolish refractory behaviour to eucapnic hyperventilation. MATTOLI *et al.* [10] studied the effect of indomethacin, 100 mg daily for 3 days, on refractory behaviour to ultrasonically distilled water (UNDW). Indomethacin abolished refractory behaviour in the group as a whole. However, two of the six subjects continued to demonstrate a reduction in airways responsiveness to UNDW after premedication with indomethacin. O'BYRNE and JONES [9] demonstrated that pretreatment with indomethacin, in an identical dosage to this study, abolished refractory behaviour to exercise in a group of seven asthmatic subjects. However, individual data on the subjects were not given and the variability of the protective effect is not known.

The present data suggest that prostaglandins, such as PGE_2 or prostacyclin, which inhibit bronchoconstriction [16, 17] may have been released during an initial hypertonic saline challenge in some individuals and that they may protect the airways from a subsequent challenge. The mechanisms for the release of prostaglandins after hyperosmolar saline challenge and the mechanisms by which prostaglandins lead to refractory behaviour are not known. Tachyphylaxis to inhaled histamine can be attenuated by premedication with indomethacin [18] and with the H_2 antagonist, cimetidine, [19] suggesting that H_2 receptor stimulation induces inhibitory prostaglandin release. The finding, that hypertonic saline challenge releases histamine, provides a potential mechanism for prostaglandin release [20]. There are several possible mechanisms by which cyclo-oxygenase products may protect the airways to a second challenge. Prostaglandin E_2 causes tachyphylaxis to exogenous histamine in canine trachealis smooth muscle, suggesting that PGE_2 may have an action on histamine receptors [21]. PGE_2 also inhibits acetylcholine release from nerve terminals in canine trachealis smooth muscle [22, 23]. *In vivo* inhalation of prostacyclin causes a significant reduction in airways responsiveness to inhaled PGD_2 and methacholine [17]. *In vivo* prostacyclin causes relaxation of precontracted guinea-pig trachea [24].

This study has shown that indomethacin does not alter basal response to hypertonic saline. Furthermore, its effect on refractoriness is variable, suggesting that there may be contributory mechanisms to the refractory period other than the release of protective prostanoid metabolites.

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