Cancer Chemother Pharmacol (1981) 5:233–236

Cancer Chemotherapy and Pharmacology

Histamine-releasing Properties of Hydroxy-9-methyl-2-ellipticinium Acetate*

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Summary. *Previous work on Hydroxy-9-methyl-2-ellipticinium acetate indicated a bronchoconstrictor activity which could be partially offset by antagonists of the H_1 histamine receptors, and the absence of any direct effect on smooth muscle.*

OH-9-CH_3-2-E at concentrations of 10 μg/ml and 500 μg/ml produced a moderate and a variable release of histamine when placed in contact with whole human blood and lung fragments, respectively.

In addition, at a dose of 3 mg/kg in the guinea pig, pulmonary airway resistance was raised and the blood histamine level lowered. A significant correlation was found between these two effects.

These results demonstrate that OH-9-CH_3-2-E possesses a histamine-releasing potency which is partly responsible for its bronchial effects, implying that precautions may have to be taken when it is used as a therapeutic agent in sensitive subjects. However, the moderate intensity of this potency has not so far precluded therapeutic use of the preparation.

Introduction

Ellipticines, and especially OH-9-CH_3-2-E, have been shown to possess antitumoral properties in studies with experimental [8–12] and human [9–13] tumours. It has been also shown by Eschalier et al. [4] that OH-9-CH_3-2-E induces bronchoconstriction in guinea pigs, this effect being partly inhibited by cyproheptadine and mepyramine, and that it does not induce direct effects on smooth muscle. Such results suggest that the compound may act by releasing endogenous mediators, including histamine.

The purpose of the present study was to investigate whether OH-9-CH_3-2-E has histamine-releasing properties. We have measured the effects of the compound on pulmonary airway resistance (PAR) and blood histamine levels in guinea pigs, and on histamine release from basophils in human blood and from mast cells in human lung tissue.

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Materials and Methods

1. In Vivo Studies on Anaesthetized Guinea Pigs

Male guinea pigs weighing from 400–600 g were divided into groups of no less than five animals, anaesthetized with urethane (1.25 g/kg IP), tracheotomized, made to lie supine, and allowed to breathe spontaneously. A catheter was inserted into the carotid artery for collection of blood samples. IV injections were given through another catheter inserted into the jugular vein. PAR was measured by the method described by Advenier et al. [1], the various parameters (intrathoracic pressure, airflow rate, and tidal volume) being continuously recorded on a Sanborn 7700 polygraphic recorder, and the pulmonary resistance calculated according to Amdur and Mead [2].

The mean initial value of PAR in our experiments was 0.45 ± 0.07 $cmH_2O/ml/s$.

In a first series of animals, the optimal non-toxic dose was determined. In a second series, the effects of a single 3 mg/kg dose of OH-9-CH_3-2-E on PAR and serum histamine levels were simultaneously studied. Five minutes before and 1, 3, 6, and 15 min after the IV injection blood samples (0.5 ml) were collected in heparinized tubes. Each sample was diluted with Tyrode solution (1.3 ml) and 4 N perchloric acid (0.2 ml) and agitated in a Vortex apparatus. The mixture was allowed to settle for 2 h, then centrifuged at 3,000 *g* for 15 min. The supernatant fluid was extracted and kept for histamine assay.

The mean initial value for histamine (as base) in our experiments was 399 ± 87 ng/ml.

2. In Vitro Studies

2.1. Whole Human Blood (Basophils.) Samples of blood from two atopic and five normal subjects were collected into heparinized tubes. For each sample four volumes of blood were mixed with one volume of Tyrode solution. Control tubes (C) for spontaneous histamine release and total histamine tubes (TH) contained 0.5 ml

0344-5704/81/0005/0233/$ 01.00

diluted blood and 0.5 ml Tyrode solution. Experimental tubes (E) contained 0.5 ml diluted blood and 0.5 ml OH-9-CH_3-2-E in concentrations ranging from 0.2–20 μg/ml. To check the ability of basophils to release histamine, the same experiments were repeated with anti-IgE serum at 1, 10, 100, and 1,000 ng Ab/ml.

The tubes, prepared in triplicate, were incubated at 37° C for 30 min. After centrifugation at 1,000 *g* and 4° C for 10 min, the supernatants of C and E tubes were deproteinized by the addition of an equal volume of 0.8 *N* perchloric acid. They were then centrifuged again at 3,000 *g* for 15 min, and the new supernatants were stored for histamine assay. The TH tubes were treated in the same manner, except for the first centrifugation, which was omitted. The results are expressed as nanograms of histamine base released by OH9$CH_3$2E. For experiments with anti-IgE serum, only the maximum percentage is given.

2.2. Human Lung Tissue (Mast Cells). Pieces from five human lungs, collected during surgery for primary lung cancer, were cut into fragments of 15–20 mg and repeatedly washed in Tyrode solution until a practically blood-free fluid was obtained. The lung fragments were randomly distributed among 20 tubes each containing 250 mg lung tissue in 4 ml Tyrode solution. Five tubes were kept as controls (C) and OH-9-CH_3-2-E in concentrations of 5, 50, and 500 μg/ml was added to 15 other tubes, each group of five tubes containing one of these concentrations. The ability of mast cells to release histamine was checked by using anti-IgE serum (100 and 1,000 ng Ab/ml).

Control and experimental tubes were treated as in the experiments already described. The results are expressed as micrograms of histamine base released by OH-9-CH_3-2-E. For experiments with anti-IgE serum, only the highest histamine value is given.

3. Histamine Determination

Histamine was assayed by a fluorimetric automated continuous flow technique [10] derived from the method of Shore. This technique makes it possible to assay 60 samples of 350 μl. The threshold of sensitivity is 200 pg histamine base per ml. The response was linear from 500 pg/ml to 5 μg/ml, with a coefficient of variation of, $\leqslant \pm 5\%$ for 0.5–2 ng/ml and of $\pm 0.5\%$–2% for higher concentrations.

Neither OH-9-CH_3-2-E nor the anti-IgE serum interfered with histamine-OPT (ortho phtalaldehyde) fluorescence.

4. Substances Used

The substances used were hydroxy-9-methyl-2-ellipticinium (acetate) dissolved in distilled water and anti-human IgE goat serum specific for ε-chains (Sodelen).

5. Statistical Analysis

A statistical analysis was performed according to Student's *t*-test in paired series.

Results

1. In Vivo Studies

1.1. Effects of OH-9-CH_3-2-E on PAR. All animals responded with an increase in PAR, but individual variations were considerable. The increase, however, was dose-related and reached significance ($P < 0.05$) at 3 mg/kg (16.6% ± 5.6%). It was not significant at 6 mg/kg (18.2% ± 12.2%), but at this dose three of the five guinea pigs developed apnoea for 50–90 s as soon as the injection was over, which suggests a toxic effect.

Kinetic studies were carried out in nine guinea pigs receiving 3 mg/kg. The PAR increased, reaching a peak (17.6% ± 2.7%) between 3 and 6 min, then gradually decreased and became non-significant at 15 min.

1.2. Effects of OH-9-CH_3-2-E on Blood Histamine. Blood histamine levels decreased to a minimum of −26.7% ± 1.7% between 3 and 6 min. They then rose again progressively but remained statistically different ($P < 0.05$) from the initial values 15 min after the injection.

There was a significant ($P < 0.05$) correlation between the increase in PAR and the decrease in blood histamine levels (Fig. 1).

2. In Vitro Studies

2.1. Whole Human Blood (Basophils). OH-9-CH_3-2-E released histamine from human basophils. This release was statistically different ($P < 0.02$) from spontaneous release (control) at 10 μg/ml, and at 0.1 and 1 μg/ml no difference was detected. Anti-IgE serum brought about a greater but more variable release (Fig. 2). Analysis of the effect in each subject shows that release occurred at concentrations of 0.1 μg/ml in 3 out of seven cases and of 10 μg/ml in seven cases. Higher concentrations could not be used on account of a haemolytic effect similar to that observed with other compounds of the same series [11]. Histamine release varied from one subject to another and was independent of both the

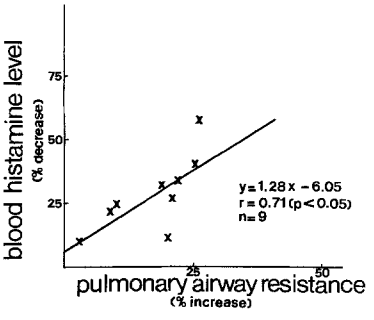

Fig. 1. Correlation between variations in pulmonary airway resistance (%) and blood histamine levels (%). *n* = number of guinea pigs

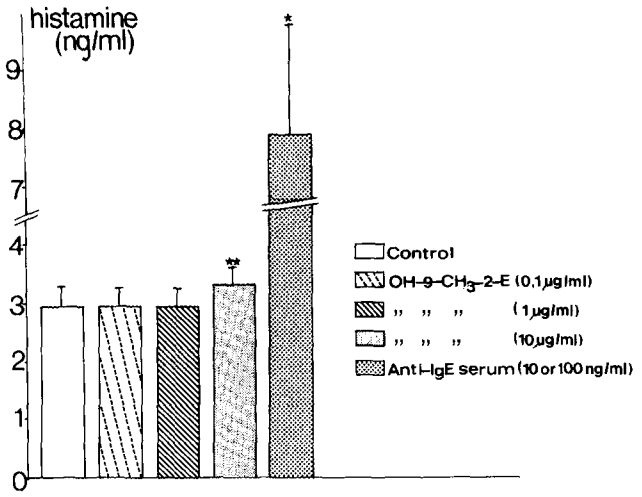

Fig. 2. Histamine release (ng/ml) from whole human blood ($n = 7$). Results are expressed as means ± SEM. *, $P < 0.05$; **, $P < 0.02$ versus control values

Table 1. Amounts of histamine (µg/g fresh tissue) released by OH-9-CH_3-2-E and by anti-IgE serum in human lung tissue

Subject no.	OH-9-CH_3-2-E (µg/ml)			Anti-IgE serum (ng/ml)
	5	50	500	(10 or 1,000[a])
1	0	0	0	0.83
2	ND[b]	0	0	3.65
3	0	0	0.50	1.44
4	ND	0.06	0.80	ND
5	0	0.11	0.04	1.01

[a] Individual concentrations resulting in maximum histamine release
[b] ND, not determined

basophil response to anti-IgE serum and the atopic or non-atopic disposition of each individual.

2.2. Human Lung Tissue (Mast Cells). The amounts of histamine released from lung mast cells increased with rising OH-9-CH_3-2-E concentrations (Table 1). At the highest concentration tested (500 µg/ml) three of five lung preparations released histamine. Here again, the response was unrelated to that obtained with anti-IgE serum and considerable individual variations were recorded.

Discussion

Previous work [4] had shown that unlike that of histamine, the bronchoconstrictor activity of OH-9-CH_3-2-E is not due to a direct action on smooth muscle, but to an indirect mechanism involving endogenous histamine.

Our in vivo studies confirm that OH-9-CH_3-2-E induces bronchoconstriction in the guinea pig, and our in vitro results show that it releases histamine, presumably from basophils in whole human blood and presumably from mast cells in human lung tissue. The response of these two types of cells is slight individual and unrelated to the immunological response observed with anti-IgE serum.

Compared with the bronchoconstriction induced by IV histamine at a dose of 3 µg/kg [3], the bronchoconstriction induced by OH-9-CH_3-2-E is moderate, which suggests that histamine is released in small amounts. This is confirmed by our in vitro experiments. It may also explain the unexpected finding that total blood histamine levels in guinea pigs decreased, since histamine released in small amounts from the circulating basophils is rapidly catabolized [14]. Such transient changes in the blood histamine pool may be compared to the decrease in skin histamine observed when histamine-releasing agents are administered in low doses, as seen with 48/80 in various animal species [7, 15]. When histamine-releasing agents are given in high doses massive histamine release from basophils and mast cells occurs, and the catabolic process is saturated. This has been demonstrated for 48/80, chlorpromazine, and morphine in rats [16] and cats [6]. It is likely, however, that the bronchoconstriction induced by OH-9-CH_3-2-E is not due to histamine alone and that other mediators released by degranulating mast cells (SRS-A, ECF-A, prostaglandins) also intervene in the process.

Nevertheless, this work shows that variable degrees of histamine release may follow OH-9-CH_3-2-E injection, this effect being confirmed by a gastric secretory activity [5] and hypotensive activity (personal results). So if similar variations exist in human species, this mechanism may contribute to side-effects during injection in some sensitive subjects, with whom care should thus be taken.

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Received June 17, 1980/Accepted February 12, 1981