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Degarelix, a novel GnRH antagonist, causes minimal histamine release compared with cetrorelix, abarelix and ganirelix in an *ex vivo* model of human skin samples

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT?

- Systemic anaphylactic reactions have been described as rare but serious adverse effects of GnRH antagonists.
- The chemical development of degarelix has devoted much attention to the elimination of this risk.
- Side-by-side comparison of the histamine releasing capacity of marketed GnRH antagonists in fresh human skin samples has not been reported yet.

WHAT THIS STUDY ADDS

- Our findings indicate considerable differences in the relative propensity of marketed GnRH antagonists to release histamine from cutaneous mast cells.
- These findings are similar but not identical to those obtained with the conventional rat peritoneal mast cell approach.
- With some further refinements the experimental set-up using human skin samples could become a useful and low-risk supplement to exploratory safety studies in clinical pharmacology.

AIMS

Early studies on gonadotrophin-releasing hormone (GnRH) antagonists pointed out histamine-mediated anaphylactic reactions as a potential adverse effect of these drug candidates. In this study we have compared the histamine-releasing potential of four approved and marketed antagonists, degarelix, cetrorelix, abarelix and ganirelix in an *ex vivo* model of human skin samples.

METHODS

Human skin samples were obtained during cosmetic plastic surgery and kept in oxygenated saline solution. The samples were incubated either without or at different concentrations of the antagonists (3, 30 or 300 μg ml^{-1} for all, except for ganirelix 1, 10 or 100 μg ml^{-1}). The drug-induced effect was expressed as the increase relative to basal release. The histamine-releasing capacity of the skin was verified by a universal histamine releaser, compound 40/80.

RESULTS

Degarelix had no significant effect on basal histamine release in the 3 to 300 μg ml^{-1} concentration range. The effect of ganirelix was moderate causing a nonsignificant increase of 81 ± 27% at the 100 μg ml^{-1} concentration. At 30 and 300 μg ml^{-1} concentrations abarelix (143 ± 29% and 362 ± 58%, respectively, $P < 0.05$) and cetrorelix (228 ± 111% and 279 ± 46%, respectively, $P < 0.05$) caused significantly increased histamine release.

CONCLUSIONS

In this *ex vivo* human skin model, degarelix displayed the lowest capacity to release histamine followed by ganirelix, abarelix and cetrorelix. These findings may provide indirect hints as to the relative likelihood of systemic anaphylactic reactions in clinical settings.

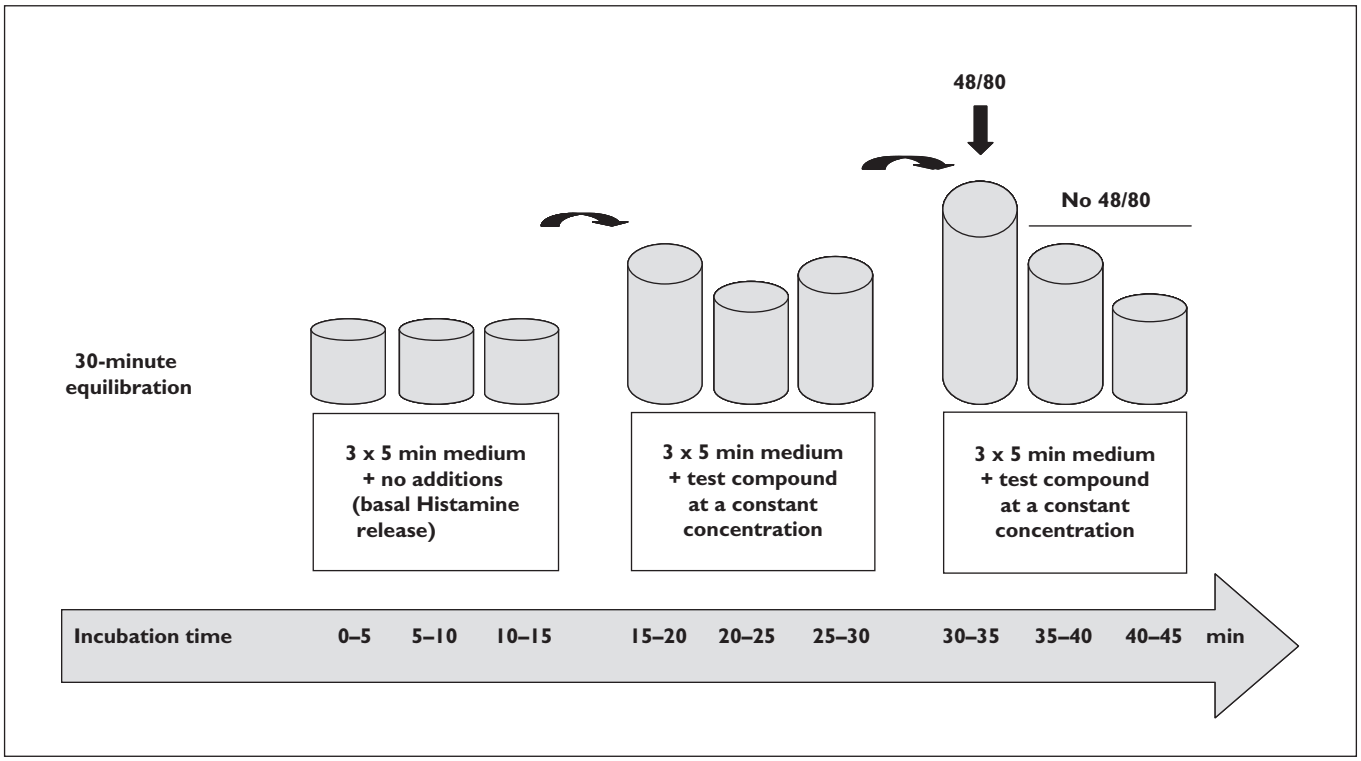

Figure 1

Schematic representation of an incubation episode. One tissue slice, from one donor, is first equilibrated for 30 min by superfusion with medium. The following incubation took place in static incubation conditions at 5 min intervals with complete exchange of media at the end of the intervals. 3× medium only for basal release estimate. 3× exposure to test compound at one concentration. 3× exposure to test compound at one concentration + 1 challenge for 5 min with compound 48/80 in incubate 7

Introduction

The bee's sting can cause devastating effects in man (and animals) and so can a subcutaneous injection of a new chemical entity. Although severe anaphylactic reactions are mainly driven by an immunological response, non-immunological mechanisms (in analogy to the direct effects of mellitin from the bee) can also cause excessive mediator release from the mast cells to induce an anaphylactoid reaction [1]. Since the latter type histamine release is of importance for clinical safety of novel therapeutic agents, various methods have been established to quantify the substance-specific potency in this regard, of which rat peritoneal mast cells are the most commonly used system. A model with *ex vivo* human skin samples that are rich in histamine and tryptase-releasing mast cells would also be of great relevance, particularly in the context of drug candidates that are administered via subcutaneous injection [2].

Gonadotrophin-releasing hormone (GnRH) antagonists represent a new class of hormonal agents, which directly block GnRH receptors and thus produce a fast sex steroid suppression. A number of these agents have undergone clinical development for the treatment of sex steroid-dependent diseases, such as uterine fibroids, endometriosis or prostate cancer. However, some of these compounds have been associated with rare but serious adverse events due to excessive histamine release from mast cells [3–7] For this reason, reduction/elimination of the histamine releasing characteristics of newer substances in this class (e.g. degarelix) has been the focus of early stage development [8].

Degarelix induces fast, profound and sustained testosterone suppression [9–11] and has recently been approved for the treatment of advanced prostate cancer by both the FDA and EMEA. In contrast to previously reported trials of other GnRH antagonists [12], no systemic anaphylactic reactions have been observed during the clinical development of degarelix in patients with prostate cancer [9, 11, 13].

In the current study, we investigated whether the aforementioned clinical side effects can be traced back to differences in the histamine-releasing potential of degarelix *vs*. three other marketed GnRH antagonists (cetrorelix, abarelix and ganirelix) using an *ex vivo* model of human skin samples.

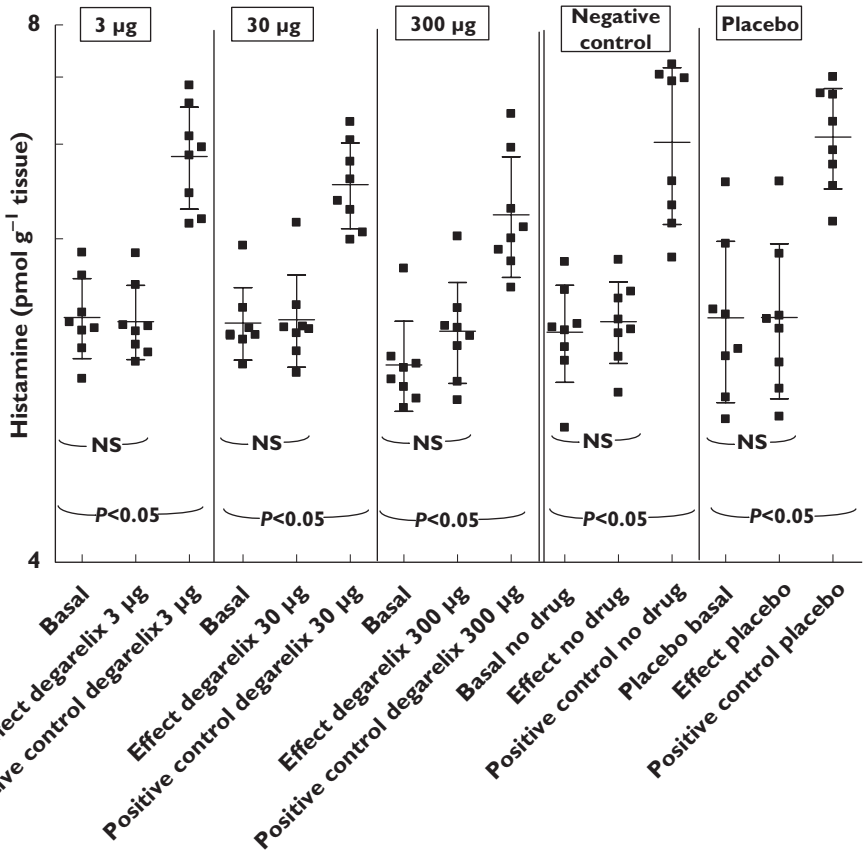

Figure 2

Effect of degarelix 3, 30 and 300 µg ml^{-1} on histamine release. Control samples of identical tissue origin were incubated without addition of the test substance. Placebo control represents the addition of 5% mannitol solution as the common diluent for test compounds. Results shown are arithmetic means of the tissue samples ± SD. Significance tests indicating NS, not significant at a 95% level and probability less than 5% = $P < 0.05$. Y-axis is shown in ln scale with antilog figures

Methods

Human skin samples

Human skin samples were obtained from individuals undergoing cosmetic surgery. Donor and ethics committee consent was obtained prior to transferring the tissue to the laboratory. The skin samples were merged and transported to the laboratory in ice-cold, oxygenated saline solution (composition in mmol l^{-1}: 125 sodium chloride, 23.8 sodium hydrogen carbonate, 5.05 glucose, 2.68 potassium chloride, 1.80 calcium chloride, 0.54 sodium dihydrogen phosphate, 0.057 ascorbic acid, 0.001 choline chloride). Upon arrival, skin strips were placed in a Petri-dish filled with oxygenated saline solution and trimmed from subcutaneous fat tissue leaving the epidermis, the dermis and part of the subcutis for testing. Subsequently, small samples of 100–150 mg were cut and fixed with a cotton thread in 2 ml organ baths. Each GnRH antagonist was tested in six to eight skin samples obtained from three to four subjects. Several pieces of skin were received from each subject and the duplicates tested were from different skin samples of the individuals.

Test substances and reagents

The GnRH antagonist test compounds were prepared as acetate salts by solid phase synthesis (minimum purity of 99%) at Ferring Research Institute Inc., San Diego, California, USA. They were dissolved in 5% mannitol solution to the required concentration and added as a bolus to the incubation medium in the organ baths.

Incubation method

The mounted skin samples were thoroughly superfused with oxygenated saline solution (2 ml min^{-1}) at 36°C for 30 min. Thereafter, they were statically incubated in 1.1 ml of saline solution. Tissue culture medium was exchanged every 5 min, and each exposure sequence included three repetitions, giving a total incubation period of 45 min for each incubate (Figure 1). Incubates 1–3 (0–15 min) were used to quantify basal or spontaneous histamine release

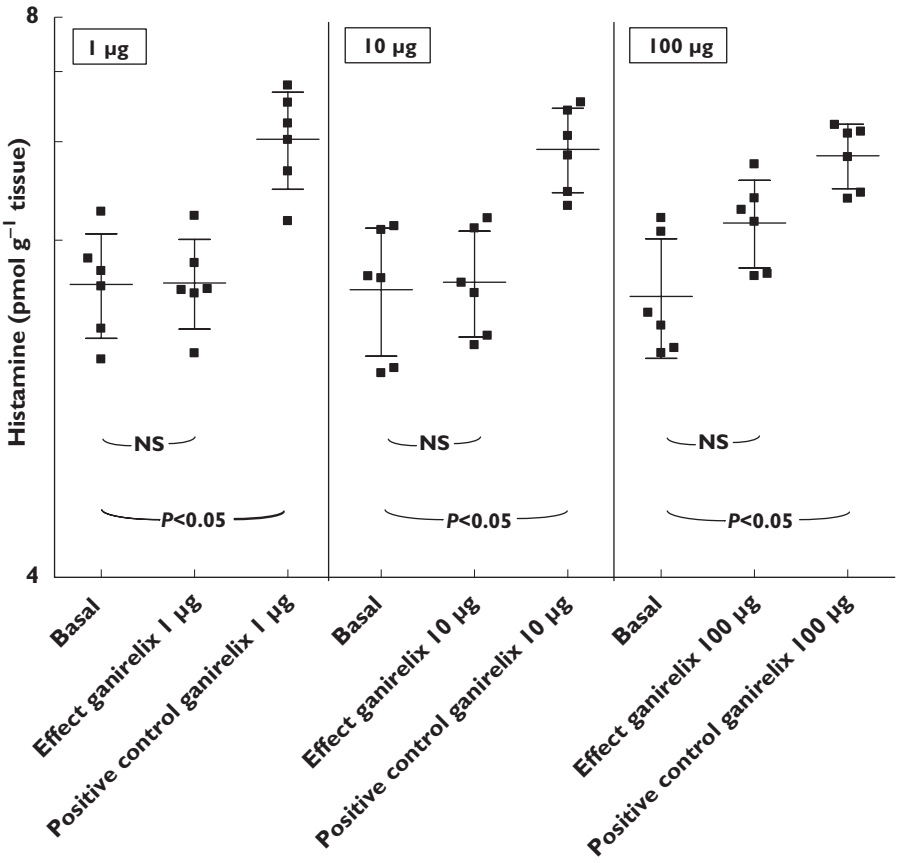

Figure 3

Effect of ganirelix 1, 10 and 100 µg ml^{-1} on histamine release. Results shown are arithmetic means of the tissue samples ± SD. Significance tests indicating NS, not significant at a 95% level and probability less than 5% = $P < 0.05$. *Y*-axis in shown in ln scale with antilog figures

from the tissue. The test substance, diluted in 5% mannitol to give final concentrations of 3, 30 or 300 µg ml^{-1} (1, 10 or 100 µg ml^{-1} for ganirelix), was then added to all subsequent incubations.

Compound 48/80, a well-known universal mast cell stimulator [14, 15], was used as a positive control (i.e. to demonstrate the actual ability of the skin strips to release histamine). The compound was used at a concentration of 30 µg ml^{-1} and added exclusively to incubate 7 of each incubation series as a 110 µl bolus (Figure 1). The subsequent incubates (8–9) were free of compound 48/80.

At the end of the complete incubation period (45 min), skin strips were dried and weighed.

Analytical procedure

Histamine content of the medium was determined after derivatization with o-phthaldialdehyde using high performance liquid chromatography and fluorometric detection. Quantification was achieved by comparison with an external histamine standard.

Calculations and statistical analysis

Histamine content of the incubation medium was normalized to 1 g dry tissue. The mean histamine content of the first three incubates (0–15 min of incubation) was regarded as the basal release. The effect of the test compound was calculated by comparing the mean of the subsequent three incubates (15–30 min) with that of the first three (0–15 min). The results are hence expressed as ratios and shown in the graphs as means with standard deviation (SD) unless otherwise indicated. Likewise, the group of the positive control was also compared with the basal release. Since the data did not show normal distribution, differences between the different series were tested after transformation of the data to natural logarithms. Means were compared using one-way analysis of variance (ANOVA) followed by Bonferroni's *t*-test as *post hoc*. Differences were considered as statistically significant if P was <0.05. All analyses was performed using the GraphPad Prism 4 Statistical software (GraphPad Software Inc, San Diego CA).

Results

Control experiments

All incubated skin samples showed detectable baseline release of histamine (Figures 2–5). Mannitol (5%), the standard diluent used for the formulation of the test sub-

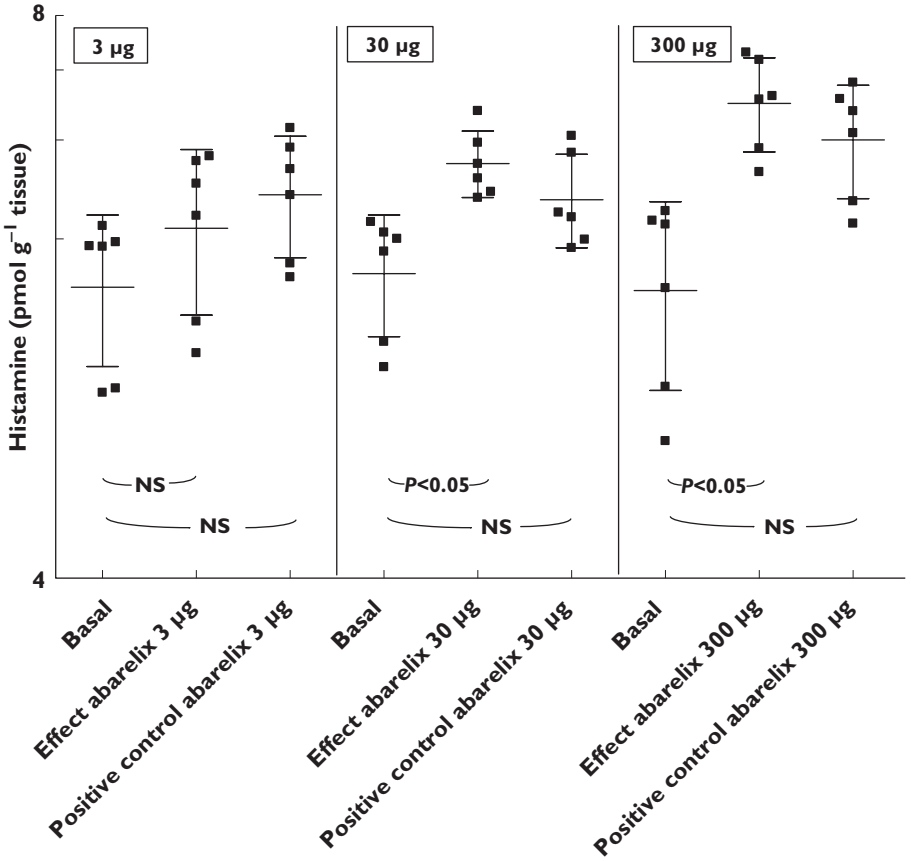

Figure 4

Effect of abarelix 3, 30 and 300 µg ml^{-1} on histamine release. Results shown are arithmetic means of the tissue samples ± SD. Significance tests indicating NS, not significant at a 95% level and probability less than 5% = $P < 0.05$. Y-axis is shown in ln scale with antilog figures

stances, did not show any direct effect on basal or stimulated histamine release (Figure 2, 'Placebo').

Effect of GnRH antagonists on histamine release

The absolute basal histamine release from the skin strips (expressed in pmol 1 g^{-1} skin tissue) and the effects expressed as ratios of stimulated and basal release are summarized in Table 1. Degarelix did not seem to elicit a significant stimulatory effect on histamine release at any of the concentrations tested (Table 1, Figure 2). Similarly, the histamine releasing capacity of ganirelix appeared to be low. Due to shortage of material the maximal concentration tested was only 100 µg ml^{-1} for ganirelix. At this maximal concentration an 81% (NS) increase in histamine-release was observed (Figure 3).

At low concentrations of abarelix (3 µg ml^{-1}), there were only slight (56%), non-significant increases in histamine release compared with basal levels. However at the two higher concentrations of 30 and 300 µg ml^{-1}, there were significant increases in histamine release ($P < 0.05$; Table 1, Figure 4). Similarly, the lowest concentration of cetrorelix (3 µg ml^{-1}) had no significant effect on histamine release, whereas the higher concentrations did elicit significant increases in histamine release corresponding to 228 and 279% for the 30 and 300 µg ml^{-1} concentrations, respectively ($P < 0.05$; Table 1, Figure 5).

The order of histamine releasing capacity from lowest to highest was degarelix (>30 µg ml^{-1}) < ganirelix (>10 µg ml^{-1}) < abarelix and cetrorelix (>3 µg ml^{-1}).

Compound 48/80

The histamine releasing effect of compound 48/80 when administered after the drug effect assessment together with the drug substance tested (Figure 1), showed overall a dependency on the prior drug exposure. Where no histamine release had occurred to the drug (e.g. to degarelix and ganirelix), the compound elicited a highly significant increase in histamine release, with slight decreases along with increasing drug concentrations. In contrast, where the drug already possessed high histamine releasing capacity at low doses (e.g. abarelix and cetrorelix), compound 48/80 could not initiate a significant additional effect as expressed as the ratio of responses during stimulation with compound 48/80 *vs.* basal conditions (Table 1, Figures 2–5).

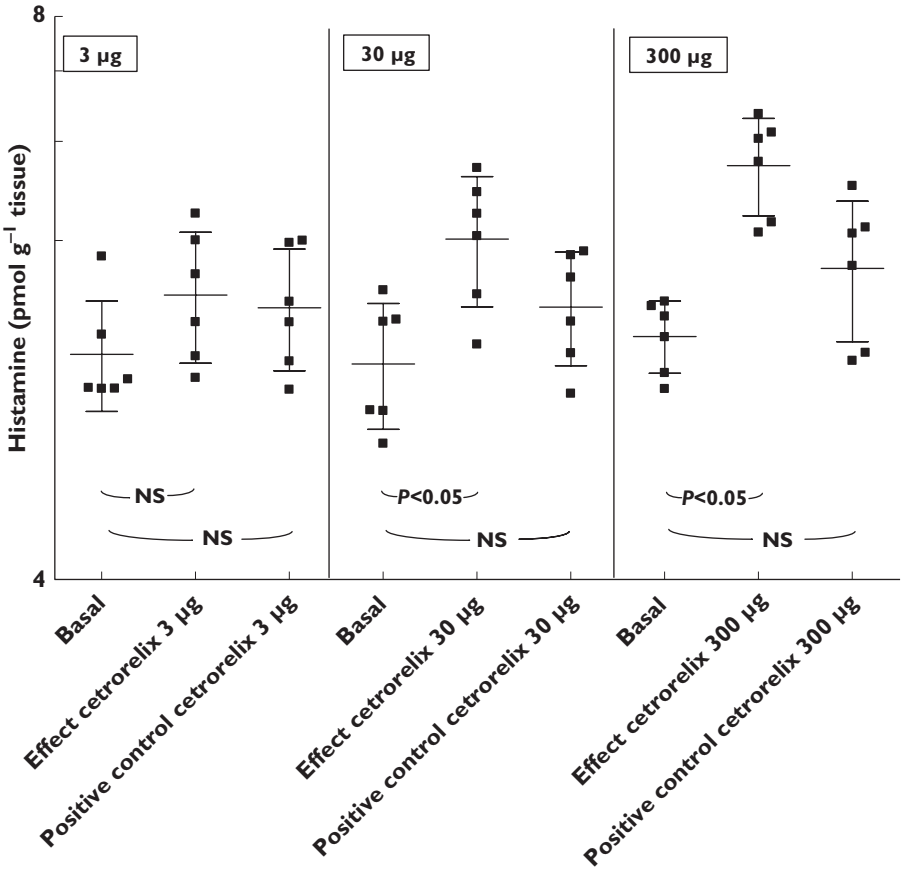

Figure 5

Effect of cetrorelix 3, 30 and 300 µg ml^{-1} on histamine release. Results shown are arithmetic means of the tissue samples ± SD. Significance tests indicating NS, not significant at a 95% level and probability less than 5% = $P < 0.05$. Y-axis is shown in ln scale with antilog figures

Discussion

The present study compared the histamine-releasing potential of several well-known GnRH antagonists utilizing an *ex vivo* experimental setup of fresh human skin samples. The main finding was the demonstration of considerable differences between the different GnRH antagonists; degarelix having the lowest (>30 µg ml^{-1}) capacity followed by ganirelix with an intermediate one (>10 µg ml^{-1}) and abarelix and cetrorelix with the highest capacity (>3 µg ml^{-1}).

The current findings in human skin tissue samples are similar to those previously noted on rat peritoneal mast cells [14]. The study showed that of the GnRH antagonists tested in our study, degarelix had the lowest and cetrorelix the highest propensity to release histamine. However, our findings obtained on human skin samples were different from those on rat peritoneal mast cells regarding the histamine releasing propensity of ganirelix and abarelix. In contrast to our findings indicating a higher propensity of abarelix to release histamine in skin samples, this GnRH antagonist showed a lower propensity to release histamine from peritoneal mast cells as compared with ganirelix. These contrasting observations point to differences in the information that can be obtained by the two different methodological approaches.

Mast cells from different species and different tissue origin may exhibit variable responses [16, 17]. Therefore it is desirable to identify and establish an appropriate predictive model that can evaluate potential adverse reactions to drugs and/or their formulations [18]. In this regard, it is also important to consider the route of administration and hence the site of primary exposure of the body. Along with these considerations, using mast cell-containing human skin explants to test drug substances that are preferably administered subcutaneously (e.g. GnRH antagonists) offers an adequate experimental approach to obtain useful information of potential clinical relevance.

The histamine-releasing potential of GnRH antagonists has been known for many years [19]. Even in the early stages of development, first-generation GnRH antagonists were frequently associated with histamine release from mast cells [20, 21]. These histamine release-mediated allergic reactions can pose significant risks to some patients

Table 1

Effect of GnRH blockers on histamine release

Compound (μg ml^{-1})	Mean basal histamine release (pmol g^{-1} ± SEM)	*n*	Effect (% increase ± SEM)	Compound 48/80 (% increase ± SEM)	Histamine liberating trend occurs at
Degarelix					>30 μg ml^{-1}
Control	229 ± 17	8	9 ± 6	476 ± 161*	
3	252 ± 19	8	–2 ± 5	283 ± 46*	
30	241 ± 19	8	3 ± 5	209 ± 37*	
300	183 ± 18	8	27 ± 6	234 ± 55*	
Placebo	282 ± 51	8	0 ± 4	416 ± 144*	
Ganirelix					>10 μg ml^{-1}
Control	328 ± 34	6	9 ± 6	166 ± 30*	
1	333 ± 39	6	5 ± 10	269 ± 104*	
10	328 ± 43	6	7 ± 6	272 ± 116*	
100	312 ± 45	6	81 ± 27	231 ± 68*	
Abarelix					<3 μg ml^{-1}
Control	257 ± 38	6	–3 ± 3	325 ± 96	
3	340 ± 47	6	56 ± 11	107 ± 18	
30	362 ± 43*	6	143 ± 29	80 ± 19	
300	350 ± 58*	6	362 ± 58	234 ± 36	
Cetrorelix					<3 μg ml^{-1}
Control	238 ± 47	6	–1 ± 2.0	299 ± 137	
3	209 ± 26	6	67 ± 28	51 ± 29	
30	198 ± 24*	6	228 ± 111	60 ± 27	
300	227 ± 16*	6	279 ± 46	68 ± 21	

*$P < 0.05$ stimulated release/basal absolute release tested.

treated with GnRH antagonists. For example, in a clinical trial involving patients with advanced, symptomatic prostate cancer, 3.7% of patients experienced an immediate-onset systemic allergic reaction within minutes after an injection of abarelix. These reactions included urticaria and pruritus as local, and hypotension and syncope as systemic reactions [6].

During the chemical phase of the development of degarelix, structural modifications were made with the aim of reducing the histamine-releasing potential while maintaining or increasing the affinity to the GnRH receptor [8]. As a result, no systemic allergic reactions have been observed with degarelix during its clinical development, which has involved more than 2000 patients [9, 11, 13, 22] The present report provides some experimental insights into these clinical differences by highlighting considerable differences in the histamine releasing capacity of skin samples when subjected to equal concentrations of degarelix or abarelix.

There are some methodological limitations to our presented approach, which are worth pointing out. For example, information about the mechanisms of action driving histamine release cannot be derived from this setup. In the herein presented setup the histamine releasing effect was calculated for each individual preparation as a percentage of the basal (unstimulated) release. Although the stimulant effect of the test compounds is normalized for the tissue mass utilized, the amount of histamine released cannot be expressed as a percentage of the total histamine content of the skin sample. The 5 min exposure to compound 48/80 at 30 min may provide useful information on the functional integrity of the skin sample, but the magnitude of the effect also depends on the test compound tested. The more potent histamine releasers, cetrorelix and abarelix diminished seemingly the histamine release to compound 48/80, whereas the poor histamine releasers, degarelix and ganirelix had minimal influence in this regard. It is possible that these interactions are also dose-dependent and most likely represent an exhaustive process. Further studies investigating the total amount of releasable histamine by, e.g. exhaustive stimulatory challenges or total tissue histamine extraction, could further refine this approach and expand its potential for the *ex vivo* characterization of drugs with histamine-releasing potential.

In summary, this study presented a simple practical approach to test the histamine-releasing potential of drugs that are administered subcutaneously in clinical settings. Using this setup, we showed that degarelix had the lowest, whereas abarelix and cetrorelix showed the highest potential to cause histamine release in fresh human skin. These *in vitro* findings are consistent with the clinical observations and provide further insights into the absence of systemic allergic reactions to degarelix in clinical trials to date. Further testing of the presently described experimental setup to supplement safety assessments in clinical pharmacological investigations seems warranted.

Competing interests

All authors are employees of Ferring Pharmaceuticals A/S.

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